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(54) Title: METHODS FOR PRODUCING IMMUNOGLOBULINS CONTAINING PROTECTION PROTEINS IN PLANTS AND THEIR USE

(57) Abstract

The immunoglobulins of the present invention are useful therapeutic immunoglobulins against mucosal pathogens such as *S. mutans*. The immunoglobulins contain a protection protein that protects the immunoglobulins in the mucosal environment. The invention also includes the greatly improved method of producing immunoglobulins in plants by producing the protection protein in the same cell as the other components of the immunoglobulins. The components of the immunoglobulin are assembled at a much improved efficiency. The method of the invention allows the assembly and high efficiency production of such complex molecules. The invention also contemplates the production of immunoglobulins containing protection proteins in a variety of cells, including plant cells, that can be selected for useful additional properties. The use of immunoglobulins containing protection proteins as therapeutic antibodies against mucosal and other pathogens is also contemplated.

Synthetic oligonucleotides

ACGACATGCTATGCAGTGTGGAGCTGCGTTTTC
CCGAAGCTTGTGTTTTTGACATGCTTTTTCTC
GATAAGCTTGTGCTCACTGCTGCTGCTGCTGA
AATTGCCAGTCACTGACGACATGCTGCTGCTG
CGAAGCTTGTGCACTGCTGCAAGCTTACA

Amplification by PCR:

Guy's 13

моро 315.



Recombinant Heavy chain

Plant G13**Plant G1/A****Plant G2/A**

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DESCRIPTIONMethods for Producing Immunoglobulins Containing
Protection Proteins in Plants and Their UseCross Reference to Related Applications

This is a continuation-in-part of co-pending application Serial No. 08/367,395 filed December 30, 1994, which is hereby incorporated by reference.

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Field of the Invention

The present invention relates to expression of immunoglobulins in plants that contain a protection protein as well as to transgenic plants that express such immunoglobulins. The therapeutic use of these immunoglobulins is also contemplated.

10

Background to the Invention

Monoclonal antibodies have great potential for numerous therapeutic purposes. The advantages of monoclonal antibody therapeutics over conventional pharmaceuticals include their exquisite selectivity, multiple effector functions, and ease of molecular manipulation such as radio-isotope labelling and other types of conjugation. A wide variety of target antigens have been used to generate specific monoclonal antibodies. See for example Therapeutic Monoclonal Antibodies, C. A. K. Borrebaeck and J.W. Larrick eds., Stockton Press, New York, 1990, and The Pharmacology of Monoclonal Antibodies, M. Rosenberg and G.P. Moore eds., Springer-Verlag, Berlin, 1994.

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One therapeutic application of monoclonal antibodies is passive immunotherapy in which the exogenously produced immunoglobulins are administered directly to the animal being treated by injection or by ingestion. To be successful, passive immunotherapy must deliver an appropriate amount of an immunoglobulin to the animal, because passive immunotherapy does not rely on an immune

30

response in the animal being treated. The immunoglobulins administered must be specific for the pathogen or molecule desired to effect treatment. One advantage of passive immunotherapy is the speed at which the antibody can be
5 contacted with the target compared to a normal immune response. Passive immunotherapy can also be used as a prophylaxis to prevent the onset of diseases or infections.

A major potential use of passive immunotherapy is in
10 combating bacterial infections. Recent emergence of antibiotic resistant bacteria make treatment of bacterial infections with passive immunotherapy desirable. Antibiotic treatment targeted to a single pathogen often involves eradication of a large population of normal
15 microbes, and this can have undesired side effects. An alternative approach has been to utilize the inherent specificity of immunoglobulins to inhibit a specific pathogenic function in very specific microbial populations. In this strategy, purified immunoglobulins
20 of the appropriate specificity would be administered in order to provide a passive barrier to pathogen invasion.

In addition, the immunoglobulins used for passive immunotherapies for example, for oral administration of immunoglobulins must meet certain requirements. First,
25 the immunoglobulin must be functional in very harsh environments, such as the gastrointestinal tract. Second, the immunoglobulin must be resistant to the actions of proteases so that it will not be degraded prior to inactivating the target.

30 Certain types of cells, including epithelial cells and hepatocytes, are capable of assembling immunoglobulin molecules which have been specifically adapted to function in harsh environments. These immunoglobulins are referred to as secretory immunoglobulins (SIg) and include both
35 secretory IgA (SIgA) and secretory IgM (SIgM). The protection provided by endogenous secretory immunoglobulins have been demonstrated. Several

mechanisms for protection from bacterial infection by secretory immunoglobulins have been proposed, including, but not limited to, direct killing, agglutination, inhibition of epithelial attachment and invasion, inactivation of enzymes and toxins, opsonization, and complement activation. In an animal, endogenously produced SIgA are exposed to very harsh environments where numerous proteases, such as intestinal and bacterial enzymes are extremely active and denaturants, such as stomach acid, are also present.

One component of secretory immunoglobulins, the secretory component, helps to protect the immunoglobulin against these inactivating agents thereby increasing the biological effectiveness of secretory immunoglobulin.

The mechanism of synthesis and assembly of these secretory immunoglobulins, such as SIgA or SIgM is extremely complex. In animal cells, secretory immunoglobulins are assembled in a process involving different cell types. Each secretory immunoglobulin is made up of immunoglobulin heavy and light chains, joining chain (J chain) and a secretory component. The immunoglobulin producing B cells make and assemble the immunoglobulin heavy and light chain together with J chain to produce dimeric or polymeric IgM or IgA. The secretory component is produced by a second type of cell, either epithelial cells or hepatocytes, and secretory immunoglobulin is assembled in and secreted from these cells. The mechanism by which these cells assemble and secrete the secretory immunoglobulin is extremely complex and requires a unique microenvironment provided, for example, by mucosal tissues. The microenvironment places the B cells that produce the polymeric immunoglobulin near the cells that assemble and secrete secretory immunoglobulin onto the mucosal surface of an animal.

The epithelial cells have a receptor, the polyimmunoglobulin receptor (pIgR), that specifically recognizes and binds polymeric immunoglobulin/containing

J chain, internalizing it and transporting it through the epithelial cell. Expressed on the basolateral cell surface, the pIgR has an N-terminal signal peptide of 18 amino acids, an extracellular polyimmunoglobulin binding
5 portion of 629 amino acids, a membrane spanning segment of 23 hydrophobic residues, and a cytoplasmic tail of 103 amino acids. The extracellular portion contains five immunoglobulin-like domains of 100-111 amino acids each and constitutes the secreted form of the molecule. See
10 for example, Mostov, Ann. Rev. Immunol., 12: 63-84 (1994) The site at which the polyimmunoglobulin receptor is cleaved to generate mature secretory component has not been accurately determined.

The polyimmunoglobulin receptor is located on the
15 basolateral surface of epithelial cells in animals. Polymeric, J chain-containing immunoglobulins produced in B cells interact with and are bound by the receptor resulting in vesicularization, transport across the epithelial cell, and ultimate secretion to the mucosal surface.
20 Transepithelial transport also involves proteolysis and phosphorylation to produce the mature SIg containing the secretory component. The close association of the required cells found in the mucosal microenvironment, specifically the B lymphocytes and epithelial cells, is
25 required for secretory immunoglobulin assembly.

The targeting of the production of immunoglobulins in transgenic organisms, such as mice, is extremely difficult and transgenic organisms made from fungus or plants do not contain the proper cell types and mucosal microenvironment
30 to produce secretory immunoglobulins. The production of large amounts of secretory immunoglobulins in transgenic organisms and cell culture has, before this invention, been impossible. One desiring to produce a secretory immunoglobulin in cell culture or a transgenic organism
35 must express the immunoglobulin heavy chain, the immunoglobulin light chain, and J chain in a B lymphocyte. To mimic the proper mucosal microenvironment a cell having

the pIgR receptor on its surface would also have to be present and be in close association with that B lymphocyte to even attempt to assemble a functional secretory immunoglobulin.

5 This elaborate process required for natural secretory immunoglobulin assembly is extremely difficult to duplicate in cell culture or transgenic organisms. Production of SIg in cell culture or transgenic organisms would require coupling the functions of cells producing
10 immunoglobulin with the functions of epithelial cells in artificial (in vitro) systems. Moreover, if the desired transgenic organism is a fungus, a bacterium, or a plant, the cell types and pathways of receptor-mediated cellular internalization, transcytosis, and secretion simply are
15 not present. Those organisms lack epithelial cells and the required mucosal microenvironment.

To date only the assembly of immunoglobulins having light, heavy and J chain within the same cell has been reported. See Carayannopoulos et al. Proc. Nat Acad. Sci., U.S.A., 91:8348-8352 (1994). However, the assembly
20 of an immunoglobulin having the additional protein component, secretory component, within a single cell has not been described.

The present invention discloses a novel method for
25 the assembly of these complex molecules. Rather than assemble the tetrameric complex at the epithelial cell surface by the interaction of a membrane bound polyimmunoglobulin receptor with immunoglobulin, we have assembled secretory immunoglobulin composed of alpha, J,
30 and kappa immunoglobulin chains associated with a protection protein derived from pIgR. This invention produces transgenic plants that assemble secretory immunoglobulins with great efficiency. The present invention makes passive immunotherapy economically
35 feasible.

Summary of the Invention

The present invention contemplates a new type of immunoglobulin molecule. Immunoglobulins of the present invention contain a protection protein in association with
5 an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain. In other embodiments, the immunoglobulin of the present invention further comprise an immunoglobulin derived light chain having at least a portion of an antigen binding domain
10 associated with the immunoglobulin derived heavy chain.

The protection proteins of the present invention give the immunoglobulins containing these protein useful properties including resistance to chemical and enzymatic degradation and resistance to denaturation. These
15 protection proteins enhanced the resistance of the immunoglobulins to environmental conditions.

The protection proteins of the proteins of the present invention comprise at least a segment of amino acid residues 1 to 606 of native polyimmunoglobulin
20 receptor (pIgR) of any species. Other useful protection proteins include protection proteins that contain portions of the pIgR molecule. For example, the protection protein may comprise all or part of: amino acids 1-118 (domain I of rabbit pIgR), amino acids 1 to 223 (domains I and II of
25 rabbit pIgR); amino acids 1 to 332 (domains I, II, III of rabbit pIgR); amino acids 1 to 441 (domains I, II, III, and IV rabbit of pIgR); amino acids 1 to 552 (domains I, II, III, IV and V of rabbit pIgR); and amino acids 1 to 606 or 1 to 627 of pIgR. Additional amino acids, derived
30 either from the pIgR sequence 653-755, or from other sources, may be included so long as they do not constitute a functional transmembrane spanning segment.

In other preferred embodiments, the immunoglobulins of the present invention have a protection protein which
35 has a first amino acid sequence which substantially corresponds to at least a portion of the amino acid residues 1 to 606 or 1 to 627 of the rabbit

polyimmunoglobulin receptor and has a second amino acid residue sequence contiguous with said first amino acid sequence, wherein said second amino acid residue sequence does not have an amino acid residue sequence corresponding
5 to the transmembrane segment of the rabbit polyimmunoglobulin receptor.

In more preferred embodiments, the second amino acid residue sequence has at least a portion of an amino acid sequence which corresponds to amino acid residues 655 to
10 755 of a polyimmunoglobulin receptor. In other preferred embodiments, the second amino acid residue is at least a portion of one or more of the following: an intracellular domain of a polyimmunoglobulin molecule, a domain of a member of the immunoglobulin gene superfamily, an enzyme,
15 a toxin, or a linker.

The present invention contemplates protection proteins which do not have an amino acid residue corresponding to the transmembrane segment of rabbit polyimmunoglobulin receptor but may have amino acid
20 residues corresponding to the intracellular domain of the rabbit polyimmunoglobulin receptor and this are deletion mutants of the receptor.

The present invention also contemplates immunoglobulins containing protection proteins which have
25 an amino acid sequence which does not contain amino acid residues of a polyimmunoglobulin receptor from a species which are analogous to amino acid residues 288 to 755 of the rabbit immunoglobulin receptor, but does contain at least a portion of the amino acid residues or the domains
30 from a polyimmunoglobulin receptor of a species which are analogous to one or more of these amino acid segments: Amino acids corresponding to amino acid residues 20-45 of the rabbit polyimmunoglobulin receptor; amino acids corresponding to or analogous to amino acid residues 1 to
35 120 of the rabbit polyimmunoglobulin receptor; amino acids corresponding to or analogous to amino acid residues numbers 120 - 230 of the rabbit immunoglobulin receptor;

amino acids corresponding to or analogous to amino acid residues numbers 230 - 340 of the rabbit polyimmunoglobulin receptor; amino acids corresponding to or analogous to amino acid residues 340 - 456 of the rabbit polyimmunoglobulin receptor; amino acids corresponding to or analogous to amino acid residues numbers 450 - 550 to 570 of the rabbit polyimmunoglobulin receptors; amino acids corresponding to or analogous to amino acid residues 550 to 570 - 606 to 627 of the rabbit polyimmunoglobulin receptor.

The protection proteins of the present invention may be derived from many species and include protection proteins derived from mammals, rodents, humans, bovine, porcine, ovine, fowl, caprine, mouse, rat, guinea pig, chicken or other bird and rabbit.

In preferred embodiments, the immunoglobulins of the present invention contain two or four immunoglobulin derived heavy chains having at least a portion of an antigen binding domain associated with the protection protein and two or four immunoglobulin derived light chains having at least a portion of an antigen binding domain bound to the each of the immunoglobulin derived heavy chains.

In other preferred embodiments, the immunoglobulins of the present invention further comprise immunoglobulin J chain bound to at least one of the immunoglobulin derived heavy chains. In preferred embodiments, the component parts of the immunoglobulins of the present invention are bound together by hydrogen bonds, disulfide bonds, covalent bonds, ionic interactions or combinations of said bonds. In other preferred embodiments, the immunoglobulin of the present invention contain protection proteins and/or immunoglobulin derived heavy, light or J chains that are free from N-linked and/or O-linked oligosaccharides.

The immunoglobulins of the present invention may be used as therapeutic immunoglobulins against, for example,

mucosal pathogen antigens. In preferred embodiments, the immunoglobulins of the present invention are capable of preventing dental caries by binding to an antigen from *S. mutans* serotypes c, e and f; and *S. sobrinus* stereotype d and g, using older nomenclature *S. mutans* a, c, d, e, f, g and h.

The present invention also contemplates a eukaryotic cell, including a plant cell, containing an immunoglobulin of the present invention. Eukaryotic cells, including plant cells, containing a nucleotide sequence encoding a protection protein and a nucleotide sequence encoding an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain is also contemplated. Eukaryotic cells, including plant cells, that additionally contain a nucleotide sequence encoding an immunoglobulin derived light chain having at least a portion of an antigen binding domain is also contemplated. In preferred embodiments, the eukaryotic cells, including plant cells, of the present invention contain nucleotide sequences that encode immunoglobulins that have an antigen binding domain is capable of binding an antigen from *S. mutans* serotypes a, c, d, e, f, and g, h (*S. mutans* serotypes c, e and f and *S. sobrinus* serotypes d and g under new nomenclature. The nucleotide sequences include RNA and appropriate DNA molecules arranged for expression.

In preferred embodiments, the plant cells of the present invention are part of a plant such as a whole plant. The present invention contemplates the use of all types of plants, both dicotyledonous and monocotyledonous including alfalfa, and tobacco.

The present invention also contemplates compositions comprising an immunoglobulin of the present invention and plant macromolecules derived from one of the plants useful in practicing the present invention. Particularly contemplated are compositions containing ribulose biphosphate carboxylase, light harvesting complex, pigments, secondary metabolites or chlorophyll and an immunoglobulin of the

present invention. Preferred compositions have an immunoglobulin concentration of between 0.001% and 99.9% mass excluding water. In more preferred embodiments, the immunoglobulin concentrations present in the composition
5 is between 0.1% and 99%. Other preferred compositions have plant macromolecules present in a concentration of between 1% and 99% mass excluding water.

The present invention also contemplates methods for making an immunoglobulin of the present invention
10 comprising introducing into a plant cell an expression vector having a nucleotide sequence encoding a protection protein operably linked to a transcriptional promoter; and introducing into the same plant cell an expression vector containing a nucleotide sequence encoding an
15 immunoglobulin derived heavy chain having at least a portion of an antigen binding domain, operably linked to a transcriptional promoter. Other methods that further include the step of introducing into the same plant cell an expression vector containing a nucleotide sequence
20 encoding an immunoglobulin derived light chain having at least a portion of an antigen binding domain, operably linked to a transcriptional promoter. Other preferred methods include also introducing into a plant cell an expression vector containing a nucleotide sequence
25 encoding an immunoglobulin J chain operably linked to a transcriptional promoter.

The present invention also contemplates methods for producing assembled immunoglobulins having heavy, light and J chains and a protection protein by introducing into
30 a eukaryotic cell nucleotide sequences operatively linked for expression to encode an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain, an immunoglobulin light chain having at least a portion of an antigen binding domain, and immunoglobulin
35 J chain, and a protection protein. The method further comprises maintaining the eukaryotic cell under conditions allowing the production and assembly of the immunoglobulin

derived heavy and light chains together with the immunoglobulin J chain and the protection protein to form an immunoglobulin containing a protection protein.

The present invention also contemplates methods of making an immunoglobulin resistant to various environmental conditions (more stable) and harsh conditions by operatively linking a nucleotide sequence encoding at least a portion of a desirable antigen binding domain derived from an immunoglobulin heavy chain to a nucleotide sequence encoding at least one domain derived from an immunoglobulin μ or α (IgM or IgA) heavy chain (or other immunoglobulin having increased stability in the environment) to form a nucleotide sequence encoding a chimeric immunoglobulin heavy chain and expressing that nucleotide sequence in a eukaryotic which also contains at least one molecule from the following list: a protection protein, an immunoglobulin derived light chain having at least a portion of an antigen binding domain and an immunoglobulin J chain. The method further comprises allowing the chimeric immunoglobulin heavy chain to assemble with the other molecule present in the same cell to form an immunoglobulin which is resistant to environmental conditions and more stable.

The large scale production of immunoglobulins of the present invention is contemplated by growing the plants of the present invention and extracting the immunoglobulins from those plants. In preferred embodiments, the method of producing therapeutic immunoglobulin compositions containing plant macromolecules includes the step of shearing under pressure a portion of a plant of the present invention to produce a pulp containing a therapeutic immunoglobulin and plant macromolecules in a liquid derived from the apoplast or symplast of the plant and solid plant derived material. Further processing steps are contemplated which include separating the solid plant derived material from the liquid and using a portion of the plant including a leaf, stem, root, tuber, flower,

fruit, seed or entire plant. The present invention contemplates the use of a mechanical device or enzymatic method which releases liquid from the apoplast or symplast of said plant followed optionally by separating using
5 centrifugation, settling, flocculation or filtration.

The present invention contemplates immunoglobulins that are chimeric and thus they contain immunoglobulin domains derived from different immunoglobulin molecules. Particularly preferred are immunoglobulins containing
10 domains from IgG, IgM and IgA.

The present invention contemplates immunoglobulins where the immunoglobulin derived heavy chain is comprised of immunoglobulin domains from two different isotopes of immunoglobulin. In preferred embodiments, the
15 immunoglobulin domains used include at least the C_H1, C_H2, or C_H3 domain of mouse IgG, IgG1, IgG2a, IgG2b, IgG3, IgA, IgE, or IgD or the C_μ domain. In other preferred embodiments, the immunoglobulin heavy chain is comprised of at least the C_μ1, C_μ2, C_μ3 or C_μ4 domain of mouse
20 IgM.

The present invention also contemplates immunoglobulin derived heavy chains made up of immunoglobulin domains include at least the C_H1, C_H2, or C_H3 domain of a human IgG, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD; or
25 least the C_μ1, C_μ2, C_μ3 or C_μ4 domain of human IgM; or the C_μ domain. The use of immunoglobulin domains derived from mammals, animals or rodents including any IgG isotype, any IgA isotype, IgE, IgM or IgD is contemplated.

The present invention also contemplates
30 tetra-transgenic organisms which are comprised of cells containing four different transgenes each encoding a different polypeptide of a multi-peptide molecule wherein at least one of those peptides is associated together to form a multi-peptide molecule. The transgenic organisms
35 contemplated by the present invention include transgenic organisms which contain as one of the four transgenes present a transgene encoding a protection protein. The

protection protein present in the transgenic organism's cells is able to assemble together with immunoglobulin heavy chains when present to form immunoglobulins which contain the protection protein.

5 In preferred transgenic organisms, the cells of the organism express four transgenes which encode an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain, an immunoglobulin derived light chain having at least a portion of an
10 antigen binding domain, an immunoglobulin J chain, and a protection protein. In other preferred transgenic organisms, the cells contain a transgene which encodes a chimeric immunoglobulin heavy chain, an immunoglobulin heavy chain derived from an IgA heavy chain, an
15 immunoglobulin derived from an IgM heavy chain or an immunoglobulin derived from some other isotype of heavy chain.

In the most preferred embodiment, the transgenic organisms of the present invention are a plant. Various
20 types and species of plants are contemplated by the present invention. In addition, the present invention also contemplates mammals which are transgenic organisms containing the various molecules of the present invention. Mammalian transgenic organisms are contemplated by the
25 present invention and include mammalian transgenic organisms which contain four transgenes encoding different polypeptides.

Brief Description of the Drawings

30 The drawings will first briefly be described.

FIGURE 1 illustrates synthetic oligonucleotides J1-J5 (restriction enzyme sites are underlined) that were used to amplify DNA fragments for Guy's 13 and alpha chain domains in the construction of hybrid IgG/A heavy chains.

35 The relative positions of the areas encoded by each oligonucleotide are shown diagrammatically. The resulting

recombinant heavy chains produced by combining various DNA fragments expressed in plants are also shown.

Detailed Description of the Invention

5 A. Definitions

Dicotyledon (dicot): A flowering plant whose embryos have two seed halves or cotyledons. Examples of dicots are: tobacco; tomato; the legumes including alfalfa; oaks; maples; roses; mints; squashes; daisies; walnuts; 10 cacti; violets; and buttercups.

Monocotyledon (monocot): A flowering plant whose embryos have one cotyledon or seed leaf. Examples of monocots are: lilies; grasses; corn; grains, including oats, wheat and barley; orchids; irises; onions and palms.

15 Lower plant: Any non-flowering plant including ferns, gymnosperms, conifers, horsetails, club mosses, liver warts, hornworts, mosses, red algae, brown algae, gametophytes, sporophytes of pteridophytes, and green algae.

20 Eukaryotic hybrid vector: A DNA by means of which a DNA coding for a polypeptide (insert) can be introduced into a eukaryotic cell.

Extrachromosomal ribosomal DNA (rDNA): A DNA found in unicellular eukaryotes outside the chromosomes, 25 carrying one or more genes coding for ribosomal RNA and replicating autonomously (independent of the replication of the chromosomes).

Palindromic DNA: A DNA sequence with one or more centers of symmetry.

30 DNA: Deoxyribonucleic acid.

T-DNA: A segment of transferred DNA.

rDNA: Ribosomal DNA.

RNA: Ribonucleic acid.

rRNA: Ribosomal RNA.

35 Ti-plasmid: Tumor-inducing plasmid.

Ti-DNA: A segment of DNA from Ti-plasmid.

Insert: A DNA sequence foreign to the rDNA, consisting of a structural gene and optionally additional DNA sequences.

Structural gene: A gene coding for a polypeptide and
5 being equipped with a suitable promoter, termination sequence and optionally other regulatory DNA sequences, and having a correct reading frame.

Signal Sequence: A DNA sequence coding for an amino acid sequence attached to the polypeptide which binds the
10 polypeptide to the endoplasmic reticulum and is essential for protein secretion.

(Selective) Genetic marker: A DNA sequence coding for a phenotypical trait by means of which transformed cells can be selected from untransformed cells.

Promoter: A recognition site on a DNA sequence or
15 group of DNA sequences that provide an expression control element for a gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Inducible promoter: A promoter where the rate of RNA
20 polymerase binding and initiation is modulated by external stimuli. Such stimuli include light, heat, anaerobic stress, alteration in nutrient conditions, presence or absence of a metabolite, presence of a ligand, microbial
25 attack, wounding and the like.

Viral promoter: A promoter with a DNA sequence substantially similar to the promoter found at the 5' end of a viral gene. A typical viral promoter is found at the 5' end of the gene coding for the p21 protein of MMTV
30 described by Huang et al., Cell, 27:245 (1981). Other examples include the promoters found in the 35S transcript of the cauliflower mosaic virus as described by Benfey et al., Science, 250:959 (1990).

Synthetic promoter: A promoter that was chemically
35 synthesized rather than biologically derived. Usually synthetic promoters incorporate sequence changes that optimize the efficiency of RNA polymerase initiation.

Constitutive promoter: A promoter where the rate of RNA polymerase binding and initiation is approximately constant and relatively independent of external stimuli. Examples of constitutive promoters include the cauliflower mosaic virus 35S and 19S promoters described by Poszkowski et al., EMBO J., 3:2719 (1989) and Odell et al., Nature, 313:810 (1985).

Regulated promoter: A promoter where the rate of RNA polymerase binding and initiation is modulated at a specific time during development, or in a specific structure of an organism or both of these types of modulation. Examples of regulated promoters are given in Chua et al., Science, 244:174-181 (1989).

Single-chain antigen-binding protein: A polypeptide composed of an immunoglobulin light-chain variable region amino acid sequence (V_L) tethered to an immunoglobulin heavy-chain variable region amino acid sequence (V_H) by a peptide that links the carboxyl terminus of the V_L sequence to the amino terminus of the V_H sequence. Generally any combination of the heavy chain and light chain antigen binding domains into the same polypeptide using a linker polypeptide to allow the binding domains to assume a useful conformation. Such combinations include V_H -Linker- V_L , V_H -Linear-Light chain, or V_L -Linear-Fd.

Single-chain antigen-binding protein-coding gene: A recombinant gene coding for a single-chain antigen-binding protein.

Polypeptide and peptide: A linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

Protein: A linear series of greater than about 50 amino acid residues connected one to the other as in a polypeptide.

Immunoglobulin product: A polypeptide, protein or protein containing at least the immunologically active portion of an immunoglobulin heavy chain and is thus

capable of specifically combining with an antigen. Exemplary immunoglobulin products are an immunoglobulin heavy chain, immunoglobulin molecules, substantially intact immunoglobulin molecules, any portion of an immunoglobulin that contains the paratope, including those portions known in the art as Fab fragments, Fab' fragment, F(ab')₂ fragment and Fv fragment.

Immunoglobulin molecule: A protein containing the immunologically active portions of an immunoglobulin heavy chain and immunoglobulin light chain covalently coupled together and capable of specifically combining with antigen.

Immunoglobulin derived heavy chain: A polypeptide that contains at least a portion of the antigen binding domain of an immunoglobulin and at least a portion of a variable region of an immunoglobulin heavy chain or at least a portion of a constant region of an immunoglobulin heavy chain. Thus, the immunoglobulin derived heavy chain has significant regions of amino acid sequence homology with a member of the immunoglobulin gene superfamily. For example, the heavy chain in an Fab fragment is an immunoglobulin derived heavy chain.

Immunoglobulin derived light chain: A polypeptide that contains at least a portion of the antigen binding domain of an immunoglobulin and at least a portion of the variable region or at least a portion of a constant region of an immunoglobulin light chain. Thus, the immunoglobulin derived light chain has significant regions of amino acid homology with a member of the immunoglobulin gene superfamily.

Antigen binding domain: The portion of an immunoglobulin polypeptide that specifically binds to the antigen. This antigen is typically bound by antigen binding domains of the immunoglobulin heavy and light chain. However, antigen binding domains may be present on a single polypeptide.

J chain: Is a polypeptide that is involved in the polymerization of immunoglobulins and transport of polymerized immunoglobulins through epithelial cells. See, The Immunoglobulin Helper: The J Chain in 5 Immunoglobulin Genes, at pg. 345, Academic Press (1989). J chain is found in pentameric IgM and dimeric IgA and typically attached via disulphide bonds. J chain has been studied in both mouse and human.

Fab fragment: A protein consisting of the portion of 10 an immunoglobulin molecule containing the immunologically active portions of an immunoglobulin heavy chain and an immunoglobulin light chain covalently coupled together and capable of specifically combining with antigen. Fab fragments are typically prepared by proteolytic digestion 15 of substantially intact immunoglobulin molecules with papain using methods that are well known in the art. However an Fab fragment may also be prepared by expressing in a suitable host cell the desired portions of immunoglobulin heavy chain and immunoglobulin light chain 20 using methods well known in the art.

F_v fragment: A protein consisting of the immunologically active portions of an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region covalently coupled together and capable of 25 specifically combining with antigen. F_v fragments are typically prepared by expressing in suitable host cell the desired portions of immunoglobulin heavy chain variable region and immunoglobulin light chain variable region using methods well known in the art.

30 Asexual propagation: Producing progeny by regenerating an entire plant from leaf cuttings, stem cuttings, root cuttings, single plant cells (protoplasts) or callus.

Self-pollination: The transfer of pollen from male 35 flower parts to female flower parts on the same plant. This process typically produces seed.

Cross-pollination: The transfer of pollen from the male flower parts of one plant to the female flower parts of another plant. This process typically produces seed from which viable progeny can be grown.

- 5 Epitope: A portion of a molecule that is specifically recognized by an immunoglobulin product. It is also referred to as the determinant or antigenic determinant.

- Chimeric immunoglobulin heavy chain: An
10 immunoglobulin derived heavy chain having at least a portion of its amino acid sequence derived from an immunoglobulin heavy chain of a different isotype or subtype or some other peptide, polypeptide or protein. Typically, a chimeric immunoglobulin heavy chain has its
15 amino acid residue sequence derived from at least two different isotypes or subtypes of immunoglobulin heavy chain.

- Transgene: A gene that has been introduced into the germ line of an animal. The gene may be introduced into
20 the animal at an early developmental stage. However, the gene could be introduced into the cells of an animal at a later stage by, for example, a retroviral vector.

- Multiple molecule: A molecule comprised of more than one peptide or polypeptide associated together by any
25 means including chemical bonds.

B. Immunoglobulins Containing Protection Proteins

- The present invention provides novel methods for producing immunoglobulin molecules containing protection
30 proteins. The immunoglobulins contain a protection protein in association with an immunoglobulin derived heavy chain that has at least a portion of an antigen binding domain.

- The protection proteins of the present invention have
35 an amino acid sequence substantially corresponding to or analogous to at least a portion of residues 1 to 627 of the amino acid residue sequence of the rabbit

polyimmunoglobulin receptor and is derived from a precursor protein that does not contain the amino acid residue sequence greater than amino acid residue 627 or analogous to amino acid residue 627 of the rabbit polyimmunoglobulin receptor. The nucleotide sequence and the amino acid sequence of the rabbit polyimmunoglobulin receptor are now and have been described by the Mostov et al., Nature, 308:37 (1984) and EMBL/Gene Bank K01291. The nucleotide sequence of the polyimmunoglobulin receptor is SEQ ID NO. 1 and the corresponding amino acid residue sequence is SEQ ID NO. 2.

The polyimmunoglobulin receptors from any species may be used as a protection protein and these protection proteins do not contain and are derived from a precursor protein that does not contain amino acids having numbers greater than the amino acid number analogous to amino acids 1-627 of the rabbit immunoglobulin sequence. In preferred embodiments, the protection protein is derived from any species and precursor protein that contains amino acids analogous to at least a portion of amino acids 1-606 of the rabbit polyimmunoglobulin receptor and does not contain amino acid residues analogous to residues 607-755 of the rabbit polyimmunoglobulin receptor.

The human polyimmunoglobulin receptor sequence has been determined and reported by Krajci et al., Eur. J. Immunol., 22:2309-2315 (1992) and Krajci et al., Biochem. Biophys. Res. Comm., 158:783-789 (1989) and EMBL/Gene Bank Accession No. X73079. The nucleotide sequence of the human polyimmunoglobulin receptor is SEQ ID NO. 3 and the corresponding amino acid residue sequence is SEQ ID NO. 4. The human polyimmunoglobulin receptor shows extensive sequence homology and has an analogous domain structure to that of the rabbit polyimmunoglobulin receptor. See, Kraehenbuhl et al., Trends in Cell Biol., 2:170 (1992). The portions of the human polyimmunoglobulin receptor which are analogous to the domains and/or amino acid

residues sequence of the rabbit polyimmunoglobulin receptor are shown in Table 1.

The rat polyimmunoglobulin receptor sequence has been determined and reported by Banting et al., FEBS Lett.,
5 254:177-183 (1989) and EMBL/Gene Bank Accession No. X15741. The nucleotide of the rat polyimmunoglobulin receptor nucleotide sequence is SEQ ID NO. 9 and the corresponding amino acid residue sequence is SEQ ID NO 10. The rat polyimmunoglobulin receptor shows extensive
10 sequence homology and has an analogous domain structure to that of the rabbit and human polyimmunoglobulin receptor. See, Kraehenbuhl et al., T. Cell Biol., 2:170 (1992). The portions of the rat polyimmunoglobulin receptor which are analogous to the domains and/or amino acid residue
15 sequence of the rabbit polyimmunoglobulin receptor are shown in Table 1.

The bovine polyimmunoglobulin receptor sequence has been determined and reported in EMBL/Gene Bank Accession No. X81371. The bovine polyimmunoglobulin receptor
20 nucleotide sequence is SEQ ID NO.5 and the corresponding amino acid residue sequence is SEQ ID NO. 6. The bovine polyimmunoglobulin receptor shows extensive sequence homology and has an analogous domain structure to that of the rabbit and human polyimmunoglobulin receptor. The
25 portions of the bovine polyimmunoglobulin receptor which are analogous to the domains and/or amino acid residues sequence of the rabbit polyimmunoglobulin receptor are shown in Table 1.

The mouse polyimmunoglobulin receptor sequence has
30 been determined and reported by Piskurich et al., J. Immunol., 150:38 (1993) and EMBL/Gene Bank U06431. The mouse polyimmunoglobulin receptor nucleotide is SEQ ID NO. 7 and the corresponding amino acid residue sequence is SEQ ID NO. 8. The mouse polyimmunoglobulin receptor shows
35 extensive sequence homology and has an analogous domain structure to that of the rabbit and human polyimmunoglobulin receptor. The portions of the mouse

polyimmunoglobulin receptor which are analogous to the domains and/or amino acid residue sequence of the rabbit polyimmunoglobulin receptor are shown in Table 1.

In addition to the above-identified nucleic acid and
5 corresponding amino acid residue sequences of the polyimmunoglobulin receptor from a variety of species, the present invention contemplates the use of a portion of a polyimmunoglobulin receptor from any species. The conserved domain structure of the polyimmunoglobulin
10 receptor between species allows the selection of analogous amino acid residue sequences within each polyimmunoglobulin receptor from different species. The present invention contemplates the use of such analogous amino acid residue sequences from any polyimmunoglobulin
15 receptor. The analogous sequences from several polyimmunoglobulin receptor amino acid sequences is as shown in Table 1.

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Table 1 Analogous Regions of the Amino Acid Residue Sequence of The Polyimmunoglobulin Receptor of Several Species. The nucleotide sequence coordinates approximately define the boundaries of the domains of molecules.

| | | Rabbit (SEQ ID NO. 2) | Bovine (SEQ ID NO. 6) | Human (SEQ ID NO. 4) | Rat (SEQ ID NO. 10) | Mouse (SEQ ID NO. 8) |
|----|----------------------|-----------------------------|-----------------------------|----------------------------|---------------------------|----------------------------|
| 5 | Immunoglobulin | | | | | |
| 10 | Binding Residues of | | | | | |
| | Domain I | 21 - 43 | ~13 - 45 | ~13 - 45 | ~13 - 45 | ~13 - 45 |
| | domain I | 1 - 118 | 1 - 120 | 1 - 120 | 1 - 120 | 1 - 120 |
| 15 | domain II | 119 - 223 | 110 - 230 | 110 - 230 | 110 - 230 | 110 - 230 |
| | domain III | 224 - 332 | 210 - 340 | 210 - 340 | 210 - 340 | 210 - 340 |
| | domain IV | 333 - 441 | 320 - 450 | 320 - 450 | 320 - 450 | 320 - 450 |
| 20 | domain V | 442 - 552 | 440 - 570 | 440 - 550 | 440 - 550 | 440 - 550 |
| | External Portions of | 553 - 606 & | 550 - 606 & | 550 - 606 & | 550 - 606 & | 550 - 606 & |
| | domain VI | 553 - 627 | 550 - 627 | 550 - 627 | 550 - 627 | 550 - 627 |
| 25 | transmembrane | | | | | |
| | segment | 630 - 652 | 625 - 660 | 625 - 660 | 625 - 660 | 625 - 660 |
| | intracellular | | | | | |
| 30 | portion | 653 - 755 | 650 - end | 650 - end | 653 - end | 653 - end |

The protection proteins of the present invention may contain substantially less than the entire amino acid residue sequence of the polyimmunoglobulin receptor. In preferred embodiments the protection protein contains at least a portion of the amino acid residues 1 to 606 of the native polyimmunoglobulin receptor of rabbit. Unlike the native polyimmunoglobulin receptor, the protection proteins of the present invention are derived from precursor proteins that do not contain the entire amino acid residue sequence greater than the amino acid residue 627 derived from the native polyimmunoglobulin receptor and thus may contain more amino acids or fewer amino acids than secretory components. In preferred embodiments, the protection proteins of the present invention do not contain the entire amino acid residue sequence greater than amino acid residue 606 of the native polyimmunoglobulin receptor of rabbit. The present invention contemplates using only portions of the native polyimmunoglobulin receptor sequence as a protection protein. In other embodiments, it is contemplated that the protection protein may end at any amino acid between amino acid residue 606 to 627, including every amino acid position between 606 and 627, such as 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626.

In preferred embodiments, a protection protein of the present invention has an amino acid sequence which corresponds to one or more of the following amino acid segments:

- 1) amino acids (AA) corresponding to AA 21-43 of domain I of the rabbit polyimmunoglobulin receptor;
- 2) amino acids (AA) corresponding to AA 1 - 118 of domain I of the rabbit polyimmunoglobulin receptor;
- 3) amino acids (AA) corresponding to AA 119 - 223 of domain II of the rabbit polyimmunoglobulin receptor;
- 4) amino acids (AA) corresponding to AA 224 - 332 of domain III of the rabbit polyimmunoglobulin receptor;

5) amino acids (AA) corresponding to AA 333 - 441 of domain IV of the rabbit polyimmunoglobulin receptor;

6) amino acids (AA) corresponding to AA 442 - 552 of domain V of the rabbit polyimmunoglobulin receptor;

5 7) amino acids (AA) corresponding to AA of 553 to 606 or 553 to 627 of domain VI of the rabbit polyimmunoglobulin receptor; and does not contain amino acid residues corresponding to AA residues 607 to 755 or 628 to 755 of the rabbit polyimmunoglobulin receptor.

10 It should be noted the exact boundary of a domain may vary within approximately 20 amino acids. However, the domain structure and boundaries will be understood by one skilled in the art.

In addition, the present invention contemplates
15 protection protein ending at the following amino acid residues of the rabbit polyimmunoglobulin receptor or at an amino acid residue which corresponds to the following residues but is in the polyimmunoglobulin receptor of another species: 580 - 605.

20 In other preferred embodiments, a protection protein has an amino acid sequence which corresponds to the amino acid sequence of a polyimmunoglobulin receptor for a particular species and which is analogous to the following amino acid segments:

25 1) amino acids (AA) corresponding to AA 21 - 43 of domain I of the rabbit polyimmunoglobulin receptor;

2) amino acids (AA) corresponding to AA 1 - 118 of domain I of the rabbit polyimmunoglobulin receptor;

3) amino acids (AA) corresponding to AA 119 - 223
30 of domain II of the rabbit polyimmunoglobulin receptor;

4) amino acids (AA) corresponding to AA 224 - 332 of domain III of the rabbit polyimmunoglobulin receptor;

5) amino acids (AA) corresponding to AA 333 - 441 of domain IV of the rabbit polyimmunoglobulin receptor;

35 6) amino acids (AA) corresponding to AA 442 - 552 of domain V of the rabbit polyimmunoglobulin receptor;

7) amino acids (AA) corresponding to AA of 553 - 606 or 553 - 627 of domain VI of the rabbit polyimmunoglobulin receptor; and does not contain amino acid residues analogous to amino acid residues 607 - 755 or 630 - 755 of the rabbit polyimmunoglobulin receptor.

In other preferred embodiments, the protection protein comprises domains I, IV, V and AA 550 - 606 or 550 - 627 of domain VI of the rabbit polyimmunoglobulin receptor or the amino acid sequence from analogous domains and regions of a polyimmunoglobulin receptor from a different species.

In other embodiments, a protection protein of the present invention has an amino acid residue sequence which substantially corresponds to at least a portion of the amino acid residues from the polyimmunoglobulin receptor of a species which are analogous to amino acid residues 1-627 of the rabbit polyimmunoglobulin receptor. This portion of the amino acid sequence would correspond to at least a portion of the extracellular domains of the receptor of that species.

In preferred embodiments, a protection protein of the present invention has an amino acid sequence which substantially corresponds to at least a portion of the amino acid residues from the polyimmunoglobulin receptor of a species which are analogous to amino acid residues 1-606 of the rabbit polyimmunoglobulin receptor.

In other preferred embodiments, a protection protein of the present invention has an amino acid residue sequence which substantially corresponds to or is analogous to (if from a species other than rabbit) at least a portion of the following amino acid residue sequences:

- 1) amino acids (AA) corresponding to AA 21 - 43 of domain I of the rabbit polyimmunoglobulin receptor;
- 2) amino acids (AA) corresponding to AA 1 - 118 of domain I of the rabbit polyimmunoglobulin receptor;

- 3) amino acids (AA) corresponding to AA 119 - 223 of domain II of the rabbit polyimmunoglobulin receptor;
- 4) amino acids (AA) corresponding to AA 224 - 332 of domain III of the rabbit polyimmunoglobulin receptor;
- 5 5) amino acids (AA) corresponding to AA 333 - 441 of domain IV of the rabbit polyimmunoglobulin receptor;
- 6) amino acids (AA) corresponding to AA 442 - 552 of domain V of the rabbit polyimmunoglobulin receptor;
- 7) amino acids (AA) corresponding to AA of 553 -
10 606 or 553 - 627 of domain VI of the rabbit polyimmunoglobulin receptor; and does not contain amino acid residues corresponding to AA 628 to 755 of the rabbit polyimmunoglobulin receptor.

In other preferred embodiments, the immunoglobulins
15 of the present invention have a protection protein which has a first amino acid sequence which substantially corresponds to at least a portion of the amino acid residues 1 to 606 or 1 to 627 of the rabbit polyimmunoglobulin receptor and has a second amino acid
20 residue sequence contiguous with said first amino acid sequence, wherein said second amino acid residue sequence does not have an amino acid residue sequence corresponding to the transmembrane segment of the rabbit polyimmunoglobulin receptor.

25 In more preferred embodiments, the second amino acid residue sequence has at least a portion of an amino acid sequence which corresponds to amino acid residues 655 to 755 of a polyimmunoglobulin receptor. In other preferred embodiments, the second amino acid residue is at least a
30 portion of one or more of the following: an intracellular domain of a polyimmunoglobulin molecule, a domain of a member of the immunoglobulin gene superfamily, an enzyme, a toxin, or a linker.

The present invention contemplates protection
35 proteins which do not have an amino acid residue corresponding to the transmembrane segment of rabbit polyimmunoglobulin receptor but may have amino acid

residues corresponding to the intracellular domain of the rabbit polyimmunoglobulin receptor and this are deletion mutants of the receptor.

In other embodiments, protection proteins of the present invention have an amino acid sequence which substantially corresponds to at least one of the extracellular domains of polyimmunoglobulin receptor of a particular species. The protection protein may have an amino acid sequence of which a segment of that amino acid sequence which substantially corresponds to an extracellular domain of the polyimmunoglobulin receptor of one species, and a different segment of that amino acid sequence may be from a second species and substantially correspond to an extracellular domain from a different species. This invention contemplates embodiments in which a protection protein has an amino acid sequence which has one amino acid sequence segment which corresponds to the amino acid sequence of the polyimmunoglobulin receptor from one species and has a second amino acid sequence within the same domain which corresponds to the amino acid and sequence of the polyimmunoglobulin receptor of a different species. Thus, the protection protein may have individual domains or portions of a particular domain that are comprised of amino acid sequences which correspond to the polyimmunoglobulin receptor from different species.

Other embodiments are contemplated in which protection protein has portions of its amino acid sequence derived from a molecule which is a member of the immunoglobulin superfamily. See, Williams and Barclay, "The Immunoglobulin Superfamily." In Immunoglobulin Genes, p. 361, Academic Press (Honjo Alt and Rabbits Eds. 1989). These derived portions may include amino acid sequences encoding peptides, domains or multiple domains from an immunoglobulin superfamily molecule.

The present invention also contemplates a nucleotide sequence encoding a protection protein which has a first nucleotide sequence encoding at least a portion of amino

acids 1-606 or 1-627 of the rabbit polyimmunoglobulin receptor nucleotide sequence and which does not have a nucleotide sequence which encodes a functional transmembrane segment 3' of the first nucleotide sequence.

5 Further preferred embodiments include a second nucleotide sequence located 3' of the first nucleotide sequence which encodes the amino acids 1-606 or 1-627 of the rabbit polyimmunoglobulin receptor sequence. This second nucleotide sequence may encode a variety of molecules

10 including portions of the intracellular domain of rabbit polyimmunoglobulin receptor or another polyimmunoglobulin receptor or a portion of an immunoglobulin superfamily molecule. In addition, embodiments are contemplated in which this second nucleotide sequence encodes various

15 effector molecules, enzymes, toxins and the like. Preferred embodiments include a second nucleotide sequence which encodes amino acid residues which correspond to amino acid residues 655 to 775 of the rabbit polyimmunoglobulin receptor or polyimmunoglobulin receptor

20 from another species.

The present invention also contemplates expression vectors containing a nucleotide sequence encoding a protection protein which has been operatively linked to for expression. These expression vectors place the

25 nucleotide sequence to be expressed in a particular cell 3' of a promoter sequence which causes the nucleotide sequence to be transcribed and expressed. The expression vector may also contain various enhancer sequences which improve the efficiency of this transcription. In

30 addition, such sequences as terminators, polydenylation (poly A) sites and other 3' end processing signals may be included to enhance the amount of nucleotide sequence transcribed within a particular cell.

In preferred embodiments, the protection protein is

35 part of an immunoglobulin that is in association with an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain. Immunoglobulin

derived heavy chains containing at least a portion of an antigen binding domain are well known in the art and have been described, for example, by Huse et al., Science, 246:1275 (1989), and by Lerner and Sorge, PCT Application
5 WO 90/14430, published November 29, 1990. The disclosure of these documents are hereby incorporated by reference.

In other embodiments, the immunoglobulins of the present invention contain a protection protein and immunoglobulin derived heavy chain and immunoglobulin
10 derived light chain that contain at least a portion of an antigen binding site in association with the immunoglobulin derived heavy chain. Immunoglobulin light chains having at least a portion of an antigen binding domain are well known in the art and are described in
15 available sources. See, for example, Early and Hood, Genetic Engineering, Setlow & Hollaender, (eds.), Vol. 3, Plenum Publishing Corp., New York (1981), pages 157-188; and Kabat et al., Sequences of Immunologic Interest, National Institutes of Health, Bethesda, Maryland (1987).
20 The disclosures of all references cited herein are hereby incorporated by reference.

The immunoglobulin components of the complex (alpha, J, kappa or lambda) can contain all or part of the full length polypeptide. Parts of these chains may be used to
25 substitute for the whole chain. For instance, the entire immunoglobulin alpha heavy chain may be replaced by the variable region and only a portion of the alpha constant region sufficient to enable assembly with the other components. Likewise, a truncated kappa or lambda chain,
30 containing only a small section of constant region can replace the full length kappa or lambda chains. The prerequisite of any complex is the ability to bind the protection protein.

In addition to truncated components, the present
35 invention contemplates the combination of different types of immunoglobulins. For example, a heavy chain constant region comprising the C_H1 and C_H2 regions of IgG followed

by the C_H2 and C_H3 regions derived from an IgA will form a stable complex containing the protection protein. This is specifically described as an example.

The immunoglobulins containing the protection
5 proteins of the present invention preferably contain at least a portion of an IgM or IgA heavy chain which allows that immunoglobulin heavy chain to bind to immunoglobulin J chain and thereby bind to the protection protein. It is contemplated that the immunoglobulin heavy chain of the
10 present invention may be comprised of individual domains selected from the IgA heavy chain or the IgM heavy chain or from some other isotype of heavy chain. It is also contemplated that an immunoglobulin domain derived from an immunoglobulin heavy chain other than IgA or IgM may be
15 molecularly engineered to bind immunoglobulin J chain and thus may be used to produce immunoglobulins of the present invention.

One skilled in the art will understand that immunoglobulins consist of domains which are approximately
20 100-110 amino acid residues. These various domains are well known in the art and have known boundaries. The removal of a single domain and its replacement with a domain of another antibody molecule is easily achieved with modern molecular biology. The domains are globular
25 structures which are stabilized by intrachain disulfide bonds. This confers a discrete shape and makes the domains a self-contained unit that can be replaced or interchanged with other similarly shaped domains. The heavy chain constant region domains of the immunoglobulins
30 confer various properties known as antibody effector functions on a particular molecule containing that domain. Example effector functions include complement fixation, placental transfer, binding to staphylococcal protein, binding to streptococcal protein G, binding to mononuclear
35 cells, neutrophils or mast cells and basophils. The association of particular domains and particular immunoglobulins isotopes with these effector functions is

well known and for example, described in Immunology, Roitt et al., Mosby St. Louis, Missouri (1993 3rd Ed.)

The immunoglobulins of the present invention may, in addition to the protection protein, contain immunoglobulin heavy chains, immunoglobulin light chains, or immunoglobulin J chain bound to the immunoglobulin derived heavy chains. In preferred embodiments, the immunoglobulin of the present invention comprises two or four immunoglobulin derived heavy chains, together with two or four immunoglobulin light chains and an immunoglobulin J chain bound to at least one of the immunoglobulin derived heavy chains. The immunoglobulin J chain is described and known in the art. See, for example, M. Koshland, The Immunoglobulin Helper: The J Chain, in Immunoglobulin Genes, Academic Press, London, Pg. 345, (1989) and Matsuuchi et al., Proc. Natl. Acad. Sci. U.S.A., 83:456-460 (1986). The sequence of the immunoglobulin J chain is available on various data bases in the United States.

The immunoglobulin of the present invention has a protection protein associated with at least an immunoglobulin derived heavy chain. This association may occur by hydrogen bonds, disulfide bonds, covalent bonds, ionic interactions or combinations of these various bonds. Typically, immunoglobulin molecules are held together by disulfide bonds between the immunoglobulin heavy chains and immunoglobulin light chains. The interaction of the protection protein with the immunoglobulin is by non-covalent or disulfide bonding.

The immunoglobulins of the present invention containing the protection protein, the immunoglobulin derived heavy chain and optionally an immunoglobulin derived light chain, and J chain are typically bonded together by one of the following: hydrogen bonds, disulfide bonds, covalent bonds, ionic interactions or combinations of these bonds. The present invention contemplates molecules in which the required portions of

the immunoglobulin heavy, light and/or J chain have been placed into a single polypeptide and function to bind antigen and protection protein. Examples of such proteins are single-chain antigen-binding proteins.

5 The present invention contemplates a method of assembling a multimeric immunoglobulin comprising the steps of: introducing into an organism a DNA segment encoding all or part of an immunoglobulin J chain, and a DNA segment encoding all or part of an immunoglobulin alpha chain, and
10 a DNA segment encoding all or part of either an immunoglobulin kappa chain or an immunoglobulin lambda chain; and introducing into the same organism a protection protein, said protection protein comprising at least a segment of the amino acid residues 1 to residue 606 of the
15 rabbit polyimmunoglobulin receptor (pIgR) amino acid residue sequence or analogous amino acid residues from other species such that the segment is derived from a precursor protein that does not contain the amino acid residues comprising a functional membrane spanning region
20 nor is the segment derived from a precursor protein in which the sequence of amino acid residues from the beginning of the membrane spanning region (approximately residue 630 of rabbit polyimmunoglobulin receptor) to the carboxyl end of the protein (approximately residue 755 of
25 the rabbit polyimmunoglobulin receptor) are fully intact. In preferred embodiments the precursor protein does not contain amino acid residues greater than 606 of the rabbit polyimmunoglobulin receptor or analogous amino acid residues from other species.

30 As is understood by those of ordinary skill in the art, a membrane spanning region or functional transmembrane segment consists of a contiguous section of amino acid residues containing from about 20 to about 30 amino acids in which none of the residues is charged, vir-
35 tually all of the residues are hydrophobic or non-polar, and the segment forms an alpha helix. A functional transmembrane segment is capable of spanning a

biomembrane. Membrane spanning regions can be bounded by charged residues. An example of a membrane spanning region of pIgR is residues 630 to 653 of the polyimmunoglobulin receptor amino acid residue sequence of rabbit.

The chains that comprise the immunoglobulin containing the protection protein may be derived from precursors containing a signal sequence at the amino terminal of the protein. Each component can thereby be synthesized into an endomembrane system where assembly occurs. In addition to a signal sequence, the various components of the complex may or may not contain additional signals for N terminal glycosylation or for various other modifications which can affect the structure of the complex. In one embodiment of the invention, the signals for glycosylation (i.e. asparagine-X-serine or threonine or the signals for O-linked glycosylation) are not present or present in more or less places within the nucleotide sequence. The resulting antibody therefore would contain no carbohydrate, which may be advantageous for applications in which carbohydrates elicit an immune response.

In preferred embodiments, the immunoglobulin of the present invention contains a protection protein associated with an immunoglobulin derived heavy chain and the protection protein is free from N-linked and/or O-linked oligosaccharides. One skilled in the art will understand that a gene coding for a polypeptide having within its amino acid residue sequence the N-linked glycosylation signal asparagine-X-serine/threonine where X can be any amino acid residue except possibly proline and aspartic acid, when introduced into a plant cell would be glycosylated via oligosaccharides linked to the asparagine residue of the sequence (N-linked). See, Marshall, Ann. Rev. Biochem., 41:673 (1972) and Marshall, Biochem. Soc. Symp., 40:17 (1974) for a general review of the polypeptide sequences that function as glycosylation

signals. These signals are recognized in both mammalian and in plant cells. One skilled in the art will understand that the N-linked glycosylation signal may be easily removed using common mutagenesis procedures to
5 change the DNA sequence encoding the protection protein of the present invention. This mutagenesis typically involves the synthesis of oligonucleotide having the N-linked glycosylation signal deleted and then preparing a DNA strand with that oligonucleotide sequence incorporated
10 into it. Such mutagenesis procedures and reagents are commercially available from many sources such as Stratagene (La Jolla, CA.).

Assembly of the individual polypeptides that form a multi-peptide molecule (for example immunoglobulin) may be
15 obtained by expressing in a single cell by directly introducing all the transgenes encoding the individual polypeptides into that cell either sequentially or all at once. The transgenes encoding the polypeptides may be present on individual constructs or DNA segments or may be
20 contained in a DNA segment or construct together with one or more other transgenes.

Assembly of these components can be by cross pollination as originally described by Mendel to produce a population of segregants expressing all chains.
25 Previous disclosures have demonstrated this to be an adequate method for the assembly and co-segregation of multimeric glycoconjugates. The disclosure of U.S. Patent No. 5,202,422 is hereby incorporated by reference and describes these methods. In a preferred embodiment of the
30 present invention, the antibody molecules contain a reduced number of glycans and antibody molecules with no glycans are contemplated.

The immunoglobulins of the present invention containing the protection protein, the immunoglobulin
35 derived heavy chain and optionally an immunoglobulin derived light chain, and J chain may contain a protection protein that is free from N-linked oligosaccharides.

The immunoglobulins of the present invention that contain the protection protein are preferably therapeutic immunoglobulins that are useful in preventing a disease in an animal. In preferred embodiments, the immunoglobulins of the present invention are therapeutic immunoglobulins which are capable of binding to mucosal pathogen antigens. In other preferred embodiments, the therapeutic immunoglobulins of the present invention are capable of preventing dental caries. In the most preferred embodiment, the immunoglobulin of the present invention containing the protection protein contains an antigen binding domain that is capable of binding to an antigen from *S. mutans* serotypes a, c, d, e, f, g and h (*S. mutans* c, e and f and *S. sobrinus* serotypes d and g under new nomenclature). Such antigen binding domains are known in the art and include, for example, the binding domains described in U.S. Patent 5,352,446, J. K-C. Ma et al., Clin. Exp. Immunol. 77:331 (1989); and J. K-C. Ma et al., Eur. J. Immunol. 24:131-138 (1994); U.S. Patent 5,352,446; U.S. Patent 4,594,244; and European Patent Publication 371 017 B1. The disclosures of these documents are hereby incorporated by reference. In preferred embodiments, the immunoglobulins of the present invention are part of a composition that has a therapeutic activity on either animals or humans. Examples of therapeutic immunoglobulins are numerous, however, we envision the most appropriate therapeutic effect to be prophylaxis for mucosal and enteric pathogens by direct oral administration of the composition derived from an edible plant.

Administration of the therapeutic composition can be before or after extraction from the plant or other transgenic organism. Once extracted the immunoglobulins may also be further purified by conventional techniques such as size exclusion, ion exchange, or affinity chromatography. In the preferred embodiment, the transgenic organism is an edible plant and administration

of the complex is by ingestion after partial purification. Plant molecules may be co-administered with the complex.

The present invention also contemplates that the relative proportion of plant-derived molecules and animal-derived molecules can vary. Quantities of specific plant
5 proteins, such as RuBisCo, or chlorophyll may be as little as 1% of the mass or as much as 99.9% of the mass of the extract, excluding water.

The present invention also contemplates the use of
10 the therapeutic plant extract containing immunoglobulins having a protection protein directly without any further purification of the specific therapeutic component, e.g. the antibody. Administration may be by topical application, oral ingestion or any other method
15 appropriate for delivering the antibody to the mucosal target pathogen. This form of administration is distinct from parenteral applications involving direct injection or comingling of the therapeutic plant extract with the blood stream.

20 The present invention also contemplates the use of the therapeutic plant extract containing immunoglobulins having a protection protein after manipulating the taste or texture of the extract. Appropriate quantities of gelling substances or flavorings could be added to enhance
25 the contact of the antibody with the target pathogen in, for example, direct oral applications.

In preferred embodiments, the immunoglobulins of the present invention are used to passively immunize an animal against a preselected ligand by contacting a composition
30 comprising an immunoglobulin containing a protection protein of the present invention that is capable of binding a preselected ligand with a mucosal surface of an animal. Passive immunization requires large amounts of antibody and for wide-spread use this antibody must be
35 inexpensive.

Immunoglobulin molecules containing protection proteins that are capable of binding a preselected antigen

can be efficiently and economically produced in plant cells. In preferred embodiments, the immunoglobulin molecule is either IgA, IgM, secretory IgM or secretory IgA or an immunoglobulin having a chimeric immunoglobulin heavy or light chain.

The immunoglobulins containing protection proteins are more resistant to proteolysis and denaturation and therefore are desirable for use in harsh environments. Contemplated harsh environments include acidic environments, protease containing environments, high temperature environments, and other harsh environments. For example, the gastrointestinal tract of an animal is a harsh environment where both proteases and acid are present. See, Kobayashi et al., Immunochemistry, 10:73 (1973).

Passive immunization of the animal using these more resistant immunoglobulins of the present invention is produced by contacting the immunoglobulin containing the protection protein with a mucosal surface of the animal. Animals have various mucosal surfaces including the lungs, the digestive tract, the nasopharyngeal cavity, the urogenital system, and the like. Typically, these mucosal surfaces contain cells that produce various secretions including saliva, lacrimal fluid, nasal fluid, tracheobronchial fluid, intestinal fluid, bile, cervical fluid, and the like.

In preferred embodiments the immunoglobulins that contain the protection protein are immunospecific for a preselected antigen. Typically, this antigen is present on a pathogen that causes a disease that is associated with the mucosal surface such as necrotizing enterocolitis, diarrheal disease, ulcers, and cancer caused by carcinogen absorption in the intestine. See e.g., McNabb and Tomasi, Ann. Revl. Microbiol., 35:477 (1981) and Lawrence et al., Science, 243:1462 (1989). Typical pathogens that cause diseases associated with a mucosal surface include both bacterial and viral

pathogens, such as E. coli., S. typhimurium, V. cholera,
H. pylori, and S. mutans. See also, European Patent
Application 484, 148 A1, published 5/6/92 and hereby
incorporated by reference. The immunoglobulins of the
5 present invention are capable of binding to these
pathogens and preventing them from causing mucosal
associated diseases.

Immunoglobulins capable of binding to S. mutans and
preventing dental caries have been described in European
10 Patent Specification 371,017 which is hereby incorporated
by reference. The disclosure of U.S. Patent No. 5,352,440
is also hereby incorporated by reference.

Therapeutic immunoglobulins of the present invention
that contain protection proteins that would be effective
15 against bacterial infection or carcinomas are
contemplated. Monoclonal antibodies with therapeutic
activity have been described in U.S. Patents 4,652,448,
4,443,549 and 5,183,756 which are hereby incorporated by
reference.

20 In preferred embodiments, the immunoglobulin of the
invention are part of a composition which is contacted
with the animal mucosal surface comprises plant material
and an immunoglobulin of the present invention that is
capable of binding a preselected ligand. The plant
25 material present may be plant cell walls, plant
organelles, plant cytoplasms, intact plant cells, viable
plants, and the like. This plant cell material is present
in a ratio from about 10,000 grams of plant material to
about 100 nanograms of immunoglobulin to about 100
30 nanograms of plant material for each 10 grams of
immunoglobulin present. In more preferred embodiments,
the plant material is present in a ratio from about 10,000
grams of plant material for each 1 gram of immunoglobulin
present to about a ratio of 100 nanograms of plant
35 material present for each gram of immunoglobulin present.
In other preferred embodiments, the plant material is
present in a ratio from about 10,000 grams of plant

material for each milligram of immunoglobulin present to about 1 milligram of plant material present for each 500 milligram of immunoglobulin present.

In preferred embodiments, the composition containing
5 the immunoglobulins of the present invention is a therapeutic composition. The preparation of therapeutic compositions which contain polypeptides or proteins as active ingredients is well understood in the art. Therapeutic compositions may be liquid solutions or
10 suspensions, solid forms suitable for solution in, or suspension in a liquid prior to ingestion may also be prepared. The therapeutic may also be emulsified. The active therapeutic ingredient is typically mixed with inorganic and/or organic carriers which are
15 pharmaceutically acceptable and compatible with the active ingredient. The carriers are typically physiologically acceptable excipients comprising more or less inert substances when added to the therapeutic composition to confer suitable consistencies and form to the composition.
20 Suitable carriers are for example, water, saline, dextrose, glycerol, and the like and combinations thereof. In addition, if desired the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents and pH buffering agents which enhance
25 the effectiveness of the active ingredient. Therapeutic compositors containing carriers that have nutritional value are also contemplated.

In embodiments in which a composition containing an immunoglobulin having a protection protein of the present
30 invention is applied to the tooth or mouth of a mammal, any convenient method may be used. Methods for applying such a composition to the teeth are well known and utilize various materials for a variety of purposes. For example, the composition may be directly applied to the tooth by
35 painting the surface of the tooth with that composition. Alternatively, the composition of the present invention may be included in a toothpaste, mouthwash, chewing gum,

lozenge or gel that will result in it being applied to the teeth. In some formulations, it may be desirable to provide for a formulation that prolongs the contact of the composition and therefore the immunoglobulin having the protection protein with the tooth surface. Formulations for this purpose are well known and include such formulations that may be placed in various dental trays that are used to cover the tooth and other dental apparatuses that are used in adjusting various conditions with the teeth.

The exact amount of a composition that must be applied to the teeth during any particular application is not critical because such treatment may be easily repeated at a given interval. For example, compositions present in toothpaste would be applied to the teeth each time that toothpaste is used, typically twice per day. For example, the order of 10 to 100 micrograms of an immunoglobulin having a protection protein can be applied to each tooth on each occasion the composition is applied to the teeth. However, this in no way should be taken as a limitation on a range that may be applied during any particular application as applications of a composition having more or less immunoglobulin of the present invention may be used without detrimental effect. The use of much lower concentrations of an immunoglobulin of the present invention would result in, at some point, a reduction in the protection provided by such formulation.

The exact formulation for the composition of the present invention may vary and will depend on the method of application to be used and the frequency of that application. In general, it may be any formulation which has an appropriate pH and which is free of material which would render the immunoglobulin having the protection protein of the present invention ineffective. For example, the compositions of the present invention may be applied as a simple aqueous solution in which the composition is disbursed at anywhere from 0.1 to 10

milligrams of immunoglobulin per 100 microliters of that solution. Generally, such a solution would be applied during dental surgery at a rate of approximately 1 to 10 microliters of the solution per tooth.

5 The formulations of the compositions of the present invention which are designed to be self-administered may vary and will be formulated taking in to account the frequency of application of the particular product in which is it used.

10 In preferred embodiments, a composition containing an immunoglobulin of the present invention comprises an immunoglobulin molecule that is immunospecific for a pathogen antigen. Pathogens are any organism that causes a disease in another organism. Particularly preferred are
15 immunoglobulins that are immunospecific for a mucosal pathogen antigen. A mucosal pathogen antigen is present on a pathogen that invades an organism through mucosal tissue or causes mucosal associated diseases. Mucosal pathogens include lung pathogens, nasal pathogens,
20 intestinal pathogens, oral pathogens, and the like. For a general discussion of pathogens, including mucosal pathogens, see, Davis et al., Microbiology, 3rd ed., Harper and Row, Hagerstown, MD (1980).

Antibodies immunospecific for a pathogen may be
25 produced using standard monoclonal antibody production techniques. See, Antibodies: A Laboratory Manual, Harlow et al., eds., Cold Spring Harbor, NY (1988). The genes coding for the light chain and heavy chain variable regions can then be isolated using the polymerase chain
30 reaction and appropriately selected primers. See, Orlandi et al., Proc. Natl. Acad. Sci., U.S.A., 86:3833 (1989) and Huse et al., Science, 246:1275 (1989). The variable regions are then inserted into plant expression vectors, such as the expression vectors described by Hiatt et al.,
35 Nature, 342:76-78 (1989).

In a preferred embodiment, the immunoglobulin of the present invention is immunospecific for an intestinal

pathogen antigen. Particularly preferred are immunoglobulins immunospecific for intestinal pathogens such as bacteria, viruses, and parasites that cause disease in the gastrointestinal tract, such as E. coli,
5 Salmonellae, Vibrio cholerae, Salmonellae typhimurium, Shigella and H. pylori.

In other preferred embodiments, the immunoglobulin containing the protection protein present in the composition is an immunoglobulin molecule that is
10 immunospecific for a dental pathogen such as Streptococcus mutans and the like. Particularly preferred are immunoglobulins immunospecific for a Streptococcus mutans antigen such as the immunoglobulin produced by hybridoma 15B2 (ATCC No. HB 8510); the hybridoma deposited as
15 European Collection of Animal cells Deposit No. 86031901; and the Guy's 13 monoclonal antibody described by Ma et al., Eur. J. Immunol., 24:131 (1994) and Smith and Lehner, Oral Micro. Immunol., 4:153 (1989).

The present invention contemplates producing passive
20 immunity in an animal, such as vertebrate. In preferred embodiments, passive immunity is produced in fish, birds, reptiles, amphibians, or insects. In other preferred embodiments passive is produced in an mammal, such as a human, a domestic animal, such as a ruminant, a cow, a
25 pig, a horse, a dog, a cat, and the like. In particularly preferred embodiments, passive immunity is produced in an adult or child mammal.

In preferred embodiments, passive immunity is produced in an animal, such as a mammal that is weaned and
30 therefore no longer nurses to obtain milk from its mother. Passive immunity is produced in such an animal by administering to the animal a sufficient amount of composition containing an immunoglobulin containing a protection protein immunospecific for a preselected ligand
35 to produce a prophylactic concentration of the immunoglobulin within the animal. A prophylactic concentration of an immunoglobulin is an amount sufficient

to bind to a pathogen present and prevent that pathogen from causing detectable disease within the animal. The amount of composition containing the immunoglobulin of the present invention required to produce a prophylactic concentrations will vary as is well known in the art with the size of the animal, the amount of pathogen present, the affinity of the particular immunoglobulin for the pathogen, the efficiency with which the particular immunoglobulin is delivered to its active location within the animal, and the like.

C. Eukaryotic Cells Containing Immunoglobulins Having A Protection Protein

The present invention contemplates eukaryotic cells, including plant cells, containing immunoglobulins of the present invention. The present invention also contemplates plant cells that contain nucleotide sequences encoding the various components of the immunoglobulins of the present invention. One skilled in the art will understand that the nucleotide sequences that encode the protection protein and the various immunoglobulin heavy and light chains and J chain will typically be operably linked to a promoter and present as part of an expression vector or cassette.

After the immunoglobulin heavy and light chain genes, and J chain genes are isolated, they are typically operatively linked to a transcriptional promoter in an expression vector.

Expression of the components in the organism of choice can be derived from an independently replicating plasmid, or from a permanent component of the chromosome, or from any piece of DNA which may transiently give rise to transcripts encoding the components. Organisms suitable for transformation can be either prokaryotic or eukaryotic. Introduction of the components of the complex can be by direct DNA transformation, by ballistic delivery into the organism, or mediated by another organism as for

example by the action of recombinant *Agrobacteria* on plant cells. Expression of proteins in transgenic organisms usually requires co-introduction of an appropriate promoter element and polyadenylation signal. In one
5 embodiment of the invention, the promoter element potentially results in the constitutive expression of the components in all of the cells of a plant. Constitutive expression occurring in most or all of the cells will ensure that precursors can occupy the same cellular
10 endomembrane system as might be required for assembly to occur.

Expression vectors compatible with the host cells, preferably those compatible with plant cells are used to express the genes of the present invention. Typical
15 expression vectors useful for expression of genes in plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers et al., Meth. in Enzymol., 153:253-277 (1987). However, several
20 other expression vector systems are known to function in plants. See for example, Verma et al., PCT Publication No. WO87/00551; and Cocking and Davey, Science, 236:1259-1262 (1987).

The expression vectors described above contain
25 expression control elements including the promoter. The genes to be expressed are operatively linked to the expression vector to allow the promoter sequence to direct RNA polymerase binding and synthesis of the desired polypeptide coding gene. Useful in expressing the genes
30 are promoters which are inducible, viral, synthetic, constitutive, and regulated. The choice of which expression vector and ultimately to which promoter a nucleotide sequence encoding part of the immunoglobulin of the present invention is operatively linked depends
35 directly, as is well known in the art, on the functional properties desired, e.g. the location and timing of protein expression, and the host cell to be transformed,

these being limitations inherent in the art of constructing recombinant DNA molecules. However, an expression vector useful in practicing the present invention is at least capable of directing the replication, and preferably also the expression of the polypeptide coding gene included in the DNA segment to which it is operatively linked.

In preferred embodiments, the expression vector used to express the genes includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II and nopaline synthase 3' nontranslated region described by Rogers et al., in Methods For Plant Molecular Biology, a Weissbach and H. Weissbach, eds., Academic Press Inc., San Diego, CA (1988). A useful plant expression vector is commercially available from Pharmacia, Piscataway, NJ.

Expression vectors and promoters for expressing foreign proteins in plants have been described in U.S. Patent Nos. 5,188,642; 5,349,124; 5,352,605, and 5,034,322 which are hereby incorporated by reference.

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracks can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Alternatively, synthetic linkers containing one or more restriction endonuclease sites can be used to join the DNA segment to the expression vector. The synthetic linkers are attached to blunt-ended DNA segments by incubating the blunt-ended DNA segments with a large excess of synthetic linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-

ended DNA molecules, such as bacteria phage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying synthetic linker sequences at their ends. These DNA segments are then cleaved with the appropriate
5 restriction endonuclease and ligated into an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the synthetic linker. Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a
10 number of sources including New England BioLabs, Beverly, MA.

The nucleotide sequences encoding the protection protein and any other of the immunoglobulins of the present invention are introduced into the same plant cell
15 either directly or by introducing each of the components into a plant cell and regenerating a plant and cross-hybridizing the various components to produce the final plant cell containing all the required components.

Any method may be used to introduce the nucleotide
20 sequences encoding the components of the immunoglobulins of the present invention into a eukaryotic cell. For example, methods for introducing genes into plants include Agrobacterium-mediated plant transformation, protoplast transformation, gene transfer into pollen, injection into
25 reproductive organs and injection into immature embryos. Each of these methods has distinct advantages and disadvantages. Thus, one particular method of introducing genes into a particular eukaryotic cell or plant species may not necessarily be the most effective for another
30 eukaryotic cell or plant species.

Agrobacterium tumefaciens-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, bypassing the need for regeneration of an intact
35 plant from a protoplast. The use of Agrobacterium-mediated expression vectors to introduce DNA into plant cells is well known in the art. See, for example, the

- methods described by Fraley et al., Biotechnology, 3:629 (1985) and Rogers et al., Methods in Enzymology, 153:253-277 (1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome as described by Spielmann et al., Mol. Gen. Genet., 205:34 (1986) and Jorgensen et al., Mol. Gen. Genet., 207:471 (1987).
- 5
- 10 Modern Agrobacterium transformation vectors are capable of replication in Escherichia coli as well as Agrobacterium, allowing for convenient manipulations as described by Klee et al., in Plant DNA Infectious Agents, T. Hohn and J. Schell, eds., Springer-Verlag, New York (1985) pp. 179-
- 15 203. Further recent technological advances in vectors for Agrobacterium-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors
- 20 described by Rogers et al., Methods in Enzymology, 153:253 (1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes.
- 25 Agrobacterium-mediated transformation of leaf disks and other tissues appears to be limited to plant species that Agrobacterium tumefaciens naturally infects. Thus, Agrobacterium-mediated transformation is most efficient in dicotyledonous plants. However, the transformation of
- 30 Asparagus using Agrobacterium can also be achieved. See, for example, Bytebier, et al., Proc. Natl. Acad. Sci., 84:5345 (1987).

In those plant species where Agrobacterium-mediated transformation is efficient, it is the method of choice

35 because of the facile and defined nature of the gene transfer. However, few monocots appear to be natural hosts for Agrobacterium, although transgenic plants have

been produced in asparagus using Agrobacterium vectors as described by Bytebier et al., Proc. Natl. Acad. Sci. U.S.A., 84:5345 (1987). Therefore, commercially important cereal grains such as rice, corn, and wheat must be transformed using alternative methods. Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments. See, for example, Potrykus et al., Mol. Gen. Genet., 199:183 (1985); Lorz et al., Mol. Gen. Genet., 199:178 (1985); Fromm et al., Nature, 319:791 (1986); Uchimiya et al., Mol. Gen. Genet., 204:204 (1986); Callis et al., Genes and Development, 1:1183 (1987); and Marcotte et al., Nature, 335:454 (1988).

Application of these systems to different plant species depends upon the ability to regenerate that particular plant species from protoplasts. Illustrative methods for the regeneration of cereals from protoplasts are described in Fujimura et al., Plant Tissue Culture Letters, 2:74 (1985); Toriyama et al., Theor Appl. Genet., 73:16 (1986); Yamada et al., Plant Cell Rep., 4:85 (1986); Abdullah et al., Biotechnology, 4:1087 (1986).

To transform plant species that cannot be successfully regenerated from protoplast, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described by Vasil, Biotechnology, 6:397 (1988). In addition, "particle gun" or high-velocity microprojectile technology can be utilized as well. Using such technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small (0.525 um) metal particles that have been accelerated to speeds of one to several hundred meters per second as described in Klein et al., Nature, 327:70 (1987); Klein et al., Proc. Natl. Acad. Sci. U.S.A., 85:8502 (1988); and McCabe et al., Biotechnology, 6:923 (1988). The metal particles penetrate through

several layers of cells and thus allow the transformation of cells within tissue explants. Metal particles have been used to successfully transform corn cells and to produce fertile, stably transformed tobacco and soybean plants. Transformation of tissue explants eliminates the need for passage through a protoplast stage and thus speeds the production of transgenic plants.

DNA can be introduced into plants also by direct DNA transfer into pollen as described by Zhou et al., Methods in Enzymology, 101:433 (1983); D. Hess, Intern. Rev. Cytol., 107:367 (1987); Luo et al., Plant Mol. Biol. Reporter, 6:165 (1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described by Pena et al., Nature, 325:274 (1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described by Neuhaus et al., Theor. Appl. Genet., 75:30 (1987); and Benbrook et al., in Proceedings Bio Expo 1986, Butterworth, Stoneham, MA, pp. 27-54 (1986).

The regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988). This regeneration and growth process includes the steps of selection of transformant cells and shoots, rooting the transformant shoots and growth of the plantlets in soil.

The regeneration of plants containing the foreign gene introduced by Agrobacterium tumefaciens from leaf explants can be achieved as described by Horsch et al., Science, 227:1229-1231 (1985). In this procedure, transformants are grown in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant species being transformed as described by Fraley et al., Proc. Natl. Acad. Sci. U.S.A., 80:4803 (1983). This procedure typically produces shoots within

two to four weeks and these transformant shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Transformant shoots that rooted
5 in the presence of the selective agent to form plantlets are then transplanted to soil to allow the production of roots. These procedures will vary depending upon the particular plant species employed, such variations being well known in the art.

10 The immunoglobulins of the present invention may be produced in any plant cell including plant cells derived from plants that are dicotyledonous or monocotyledonous, solanaceous, alfalfa, legumes, or tobacco.

Transgenic plants of the present invention can be
15 produced from any sexually crossable plant species that can be transformed using any method known to those skilled in the art. Useful plant species are dicotyledons including tobacco, tomato, the legumes, alfalfa, oaks, and maples; monocotyledons including grasses, corn, grains,
20 oats, wheat, and barley; and lower plants including gymnosperms, conifers, horsetails, club mosses, liver warts, horn warts, mosses, algae, gametophytes, sporophytes of pteridophytes.

The plant cells of the present invention may in
25 addition to the protection protein and the immunoglobulin derived heavy chain also contains a nucleotide sequence encoding an immunoglobulin derived light chain having at least a portion of an antigen binding domain.

The plant cells of the present invention may have an
30 antigen binding domain that is capable of binding an antigen from S. mutans serotypes a, c, d, e, f, g, and h (*S. mutans* serotypes c, e, and f; and *S. sobrinus* serotypes d and g under new nomenclature) on the immunoglobulin derived heavy and light chains. T h e
35 antigen binding domain present in these plant cells also can be able to bind to the responsible mucosal pathogens and prevent dental caries.

The plant cells of the present invention may be part of a plant and make up one of the following types of plants: dicotyledonous, monocotyledonous, solanaceous, alfalfa, tobacco or other type of plant.

5

D. Compositions Containing Immunoglobulins Having Protection Proteins

The present invention contemplates compositions of matter that comprise immunoglobulins of the present
10 invention and plant macromolecules. Typically these plant macromolecules are derived from any plant useful in the present invention. The plant macromolecules are present together with an immunoglobulin of the present invention for example, in a plant cell, in an extract of a plant
15 cell, or in a plant. Typical plant macromolecules associated with the immunoglobulins of the present invention in a composition are ribulose biphosphate carboxylase, light harvesting complex, (LH6) pigments, secondary metabolites or chlorophyll. The compositions of
20 the present invention have an immunoglobulin of the present invention present in a concentration of between 1% and 99% mass excluding water. Other preferred compositions include compositions having the immunoglobulins of the present invention present at a
25 concentration of between 1% and 50% mass excluding water. Other preferred compositions include immunoglobulins at a concentration of 1% to 25% mass excluding water.

The compositions of the present invention contain plant macromolecules at a concentration of between 1% and
30 99% mass excluding water. Typically the mass present in the composition will consist of plant macromolecules and immunoglobulins of the present invention. When the immunoglobulins of the present invention are present at a higher or lower concentration the concentration of plant
35 macromolecules present in the composition will vary inversely. In preferred embodiments the composition of plant macromolecules are present in a concentration of

between 50% and 99% mass excluding water. In the most preferred compositions, the plant macromolecules are present in a concentration of between 75% and 99% mass excluding water.

5 The present invention contemplates a composition of matter comprising all or part of the following: an IgA heavy chain, a kappa or lambda chain, a J chain. These components form a complex and are attached to the protection protein as defined earlier. The composition
10 also contains molecules derived from a plant. This composition may also be obtained after an extraction process yielding functional antibody and plant-derived molecules.

 The extraction method comprises the steps of applying
15 a force to a plant containing the complex whereby the apoplastic compartment of the plant is ruptured releasing said complex. The force involves shear, in dyn/cm², as the primary method of releasing the apoplastic liquid.

 The whole plant or plant extract contains an
20 admixture of antibody and various other macromolecules of the plant. Among the macromolecules contained in the admixture is ribulose biphosphate carboxylase (RuBisCo) or fragments of RuBisCo. Another macromolecule is LHCP. Another molecule is chlorophyll.

25 Shear force is a useful component of the overall force applied to the plant for disruption of apoplastic spaces. Other types of force may also be included to optimize the effects of shear. Direct pressure, for example, measured in lbs/in², may enhance the effects of
30 the apparatus used to apply shear. Commonly used homogenization techniques which are not appropriate for antibody extraction involve the use of high speed blades or cylinders which explosively destroy all plant structures.

35 The compositions of the present invention may contain an immunoglobulin of the present invention and plant molecules that are derived from a dicotyledonous,

monocotyledonous, solanaceous, alfalfa, tobacco or other plant. The plant molecules present in the compositions of the present invention can be ribulose biphosphate carboxylase, light harvesting complex, pigments, secondary metabolites, chlorophyll or other plant molecules.

Other useful methods for preparing composition containing immunoglobulins having protection protein include extraction with various solvents and application of vacuum to the plant material. The compositions of the present invention may contain immunoglobulins of the present in a concentration of between 1% and 99% mass excluding water. The compositions of the present invention may contain plant macromolecules in a concentration of between 1% and 99% mass excluding water.

Therapeutic compositions containing immunoglobulins of the present invention and plant macromolecules may be produced by processing a plant of the present invention by shearing under pressure a portion of that plant to produce a pulp containing the therapeutic immunoglobulin and plant macromolecules in a liquid derived from the apoplast or symplast of the plant which also contains the solid plant derived material. Further processing may be accomplished by separating the solid plant derived material from the plant derived liquid containing the immunoglobulins of the present invention. The starting material for such a process may include plant leaves, stem, roots, tubers, seeds, fruit or the entire plant. Typically, this processing is accomplished by a mechanical device which releases liquid from the apoplast or symplast of the plant. Additional processing steps may include separation of the solid plant derived material from the liquid using centrifugation settling flocculation or filtration. One skilled in the art will understand that these separation methods result in removing the solid plant derived material from the liquid including the immunoglobulins of the present invention. The methods of the present invention may produce immunoglobulins containing a

protection protein and an immunoglobulin derived heavy chain that is comprised of domains or portions of immunoglobulin alpha chain and immunoglobulin gamma chain. The methods of the present invention may produce
5 immunoglobulins containing a protection protein and an immunoglobulin derived light chain that is comprised of domains or portions of immunoglobulin kappa or lambda chain.

The methods of the present invention are operable on
10 plant cells or part of a plant. The methods of the present invention may also include methods that further comprise growing the plant. The methods of the present invention may be applied to any plant including dicotyledonous, monocotyledonous, solanaceous, leguminous,
15 alfalfa or tobacco plant. The methods of the present invention may be used to extract immunoglobulins from a portion of the plant such as a leaf, stem, root, tuber, seeds, fruit or entire plant. The methods of the present invention may use a mechanical device to shear the plants
20 to release liquid from the apoplast or symplast of the plant. The plant pulp of the present invention may be separated to remove the solid plant material using one of the following methods: centrifugation, settling, flocculation or filtration.

25

E. Methods of Producing Immunoglobulins Containing Protection Proteins

The present invention contemplates methods of producing an immunoglobulin containing a protection
30 protein comprising the steps of:

(a) Introducing into the plant cell an expression vector containing a nucleotide sequence encoding a protection protein operatively linked to a transcriptional promoter; and

35 (b) Introducing into the same plant cell an expression vector containing a nucleotide sequence encoding an immunoglobulin derived heavy chain having at

least a portion of an antigen binding domain operatively linked to a transcriptional promoter.

The methods of the present invention optionally include introducing into the plant cell containing the
5 expression vector with the nucleotide sequences for the protection protein and the immunoglobulin derived heavy chain a nucleotide sequence encoding an immunoglobulin derived light chain at least having a portion of an antigen binding domain operatively linked to a
10 transcriptional promoter. Methods are also contemplated that introduce into a cell that already contains nucleotide sequences and promoters operatively linked to encode a protection protein and an immunoglobulin heavy chain and an immunoglobulin light chain, a promoter
15 operatively linked to a nucleotide sequence encoding J chain. This results in a cell containing the nucleotide sequences operatively linked to promoters for an immunoglobulin heavy chain and an immunoglobulin light chain, J chain and a protection protein.

20 The plant cells of the present invention may be present as part of a plant that is capable of growth. Particularly useful plants for this invention include dicotyledonous, monocotyledonous, solanaceous, legumes, alfalfa, tomato, and tobacco plants.

25 The methods of the present invention include producing an assembled immunoglobulin having heavy, light and J chains and a protection protein within a eukaryotic cell. This eukaryotic cell is produced by introducing into that cell nucleotide sequences operatively linked for
30 expression encoding an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain, an immunoglobulin derived light chain having at least a portion of an antigen binding domain, an immunoglobulin J chain, and a protection protein. These nucleotide
35 sequences are operatively linked for expression by attaching appropriate promoters to each individual nucleotide sequence or to more than one nucleotide

sequence thereby placing two nucleotide sequences encoding various molecules in tandem.

The eukaryotic cell produced by the present methods which contains these nucleotide sequences encoding the
5 immunoglobulin heavy, light and J chains and the protection protein is maintained under conditions which allow those molecules to reproduce and assemble into an immunoglobulin which contains the protection proteins of the present invention.

10 The present invention also contemplates methods for making a particular immunoglobulin or antigen binding domain or domains of an immunoglobulin resistant to environmental conditions and more stable by operatively linking a nucleotide sequence encoding at least a portion
15 of an antigen binding domain derived from an immunoglobulin heavy chain to a nucleotide sequence encoding at least one domain derived from an immunoglobulin α or μ heavy chain to form a nucleotide sequence encoding a chimeric immunoglobulin heavy chain.
20 That nucleotide sequence encoding the chimeric immunoglobulin heavy chain is expressed in a eukaryotic cell which also contains at least one other molecule such as a protection protein, an immunoglobulin derived light chain having at least a portion of an antigen binding
25 domain and an immunoglobulin J chain. In preferred embodiments, the cell contains all of the molecules including an immunoglobulin derived light chain having an antigen binding domain which is complementary to the antigen binding domain present on the immunoglobulin
30 derived heavy chain. This method allows the chimeric immunoglobulin heavy chain to assemble with at least one other molecule, for example, the immunoglobulin derived light chain having the complementary antigen binding domain and an immunoglobulin J chain and the protection
35 protein to form an immunoglobulin containing the protection protein which is resistant to environmental conditions.

These immunoglobulins are resistant to environmental conditions and thus more stable when subjected to elevated or reduced temperatures, high or low pH, high ionic or low ionic concentrations proteolytic enzymes and other harsh
5 conditions. Such harsh conditions are typically found in the environment within natural water sources, within the human body, for example within the gut and on mucosal surfaces, and on the surface of an animal such as a mammal.

10

F. Chimeric Immunoglobulins Containing Protection Proteins

The present invention contemplates immunoglobulins containing a protection protein in which the
15 immunoglobulin domains comprising the heavy and light chain are derived from different isotopes of either heavy or light chain immunoglobulins. One skilled in the art will understand that using molecular techniques these domains can be substituted for a similar domain and thus
20 produce an immunoglobulin that is a hybrid between two different immunoglobulin molecules. These chimeric immunoglobulins allow immunoglobulins containing protection proteins to be constructed that contain a variety of different and desirable properties that are
25 conferred by different immunoglobulin domains.

The present invention also contemplates chimeric immunoglobulins, including heavy, light and J chain which contain less than an entire domain derived from a different molecule. The same molecular techniques may be
30 employed to produce such chimeric immunoglobulins.

In preferred embodiments, the immunoglobulins of the present invention contain at least the C_H1, C_H2, C_H3, domain of mouse IgG, IgG1, IgG2A, IgG2B, IgG3, IgA, IgE, or IgD. Other preferred embodiments of the present invention
35 contain immunoglobulin domains that include at least the C_μ1, C_μ2, C_μ3, or C_μ4 domain of mouse IGM. Preferred

immunoglobulins include immunoglobulins that contain the domains of C ϵ 2, C ϵ 3, and C ϵ 4 of mouse immunoglobulin IGE.

The present invention also contemplates chimeric immunoglobulins derived from human immunoglobulins. These
5 chimeric immunoglobulins contain domains from two different isotopes of human immunoglobulin. Preferred immunoglobulins include immunoglobulins that contain immunoglobulin domains including at least the C μ 1, C μ 2, or C μ 3 of human IgG, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgE,
10 or IgD. Other preferred immunoglobulins include immunoglobulins that contain domains from at least the C μ 1, C μ 2, C μ 3, or C μ 4 domain of human IgM or IgE. The present invention also contemplates immunoglobulins that contain immunoglobulin domains derived from at least two different
15 isotopes of mammalian immunoglobulins. Generally, any of the mammalian immunoglobulins can be used in the preferred embodiments, such as the following isotopes: any isotype of IgG, any isotype of IgA, IgE, IgD or IgM. The immunoglobulins of the present invention contained at
20 least one of the constant region domains from two different isotopes of mammalian immunoglobulin.

The present invention also contemplates immunoglobulins that contain immunoglobulin domains derived from two different isotopes of rodent
25 immunoglobulin. The isotopes of rodent immunoglobulin are well known in the art. The immunoglobulins of the present invention may contain immunoglobulin derived heavy chains that include at least one of the following immunoglobulin domains: the C μ 1, C μ 2, or C μ 3 domain of a mouse IgG, IgG1,
30 IgG2a, IgG2b, IgG3, IgA, IgE, or IgD; the C μ 1, C μ 2, C μ 3, C μ 4 domain of mouse IgE or IgM; the C μ 1, C μ 2, or C μ 3 domain of a human IgG, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD; the C μ 1, C μ 2, C μ 3, C μ 4 domain of human IgM or IgE; the C μ 1, C μ 2, or C μ 3 domain of an isotype of mammalian IgG, an
35 isotype of IgA, IgE, or IgD; the C μ 1, C μ 2, C μ 3, C μ 4 domain of a mammalian IgE or IgM; the C μ 1, C μ 2, or C μ 3 domain of an isotype of rodent IgG, IgA, IgE, or IgD; the C μ 1, C μ 2,

C_H3, C_H4 domain of a rodent IgE or IgM; the C_H1, C_H2, or C_H3 domain of an isotype of animal IgG, an isotype of IgA, IgE, or IgD; and the C_H1, C_H2, C_H3, C_H4 domain of an animal IgE or IgM. The present invention also contemplates the replacement or addition of protein domains derived from molecules that are members of the immunoglobulin superfamily. The molecules that belong to the immunoglobulin superfamily have amino acid residue sequence and nucleic acid sequence homology to immunoglobulins. The molecules that are part of the immunoglobulin superfamily can be identified by amino acid or nucleic acid sequence homology. See, for example, p. 361 of Immunoglobulin Genes, Academic Press (1989).

Tetratransgenic Organisms:

The present invention also contemplates a tetratransgenic organism which is comprised of cells having incorporated into the nucleic acid of that cell or plant within the cell four different transgenes, each encoding a different polypeptide. These transgenes are different in that the messenger RNA and polypeptides produced from that transgene are different from the messenger RNA and polypeptides produced from the other of the four transgenes. Thus, the number of transgenes referred to in the present invention does not include multiple copies of the same transgene as is commonly found in transgenic organisms. The present invention is directed to transgenic organisms having four transgenes which are not identical copies of other transgenes. The present invention does not exclude the possibility that each of the four different transgenes may be present in multiple copies. However, at least four separate transgenes that are different are present within the cells of the transgenic organism.

In addition, the present invention contemplates that four different transgenes are related in that the transgenes encode a polypeptide that is part of a multipolypeptide molecule. Therefore, the present

invention contemplates that each individual polypeptide chain of a multi-peptide molecule would be present on a transgene within a cell of the transgenic organism. The expression of each individual different polypeptide of the multi-peptide molecule allows the different polypeptides to associate together to form the multi-peptide molecule within the transgenic animal's cells. Thus, the present invention does not include within the four different transgenes in each individual cell, transgenes which encode polypeptides which do not associate together to perform a multi-peptide molecule. Examples of such transgenes encoding molecules that do not associate together are polypeptides for antibiotic resistance such as kanamycin or neomycin or thymidine kinase.

In preferred embodiments, the transgenes present within a transgenic organism of the present invention encode the following four different polypeptides: a protection protein; an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain; an immunoglobulin derived light chain having at least a portion of an antigen binding domain; and an immunoglobulin J chain. In other preferred embodiments, one of the transgenes present in the transgenic organism encodes a chimeric immunoglobulin heavy, light or J chain. In other preferred embodiments, a transgene of the transgenic organisms of the present invention encode either an immunoglobulin heavy chain derived at least in part from an IgA or a IgM immunoglobulin. Other preferred embodiments include transgenic organisms containing transgenes which encode at least a portion of the amino acid sequence derived from an immunoglobulin heavy chain derived from either an IgA or IgM immunoglobulin heavy chain.

The present invention contemplates transgenic organisms including mammals, plants, rodents, reptiles, insects, amphibians, fishes or other organisms. In preferred embodiments, the transgenic organism of the

present invention is a plant or a mammal. Methods of producing such organisms are well known. See, i.e., U.S. Patents 4,736,866; 4,607,388; 4,870,009 and 4,873,191 which are hereby incorporated by reference.

5 The present invention also contemplates immunoglobulin that contain immunoglobulin derived heavy or immunoglobulin derived light chains that contain immunoglobulin domains which have been engineered to make those domains less immunogenic in a particular species.
10 Typically, the immunoglobulin molecule is engineered as to be "humanized" in that it appears to be a human immunoglobulin even though derived from various other species.

15 Examples

 The following examples illustrate the disclosed invention. These examples in no way limit the scope of the claimed invention.

20 1. Construction of DNA Vectors For Expression of Antibodies in Plants.

 a. Isolation of the Nucleotide Sequences Encoding the Guy's 13 Immunoglobulin

 Molecular cloning of the gamma and kappa chains of the Guy's 13 anti-S. mutans antibody was done by the
25 procedures described in Ma et al., Eur. J. Immunol., 24:131 (1994). Briefly, mRNA was extracted from the Guy's 13 hybridoma cell line and converted to the cDNA by standard procedures. The cDNA was then amplified with the use of a pair of oligonucleotides specifically
30 complementary to either the gamma or kappa cDNA. Amplification was catalyzed by Taq 1 polymerase using a thermal cycler as described. The amplified cDNAs were then digested with the appropriate restriction endonucleases and ligated into the corresponding
35 restriction site in a standard plant expression vector. Numerous examples of such vectors have been reported in

the literature and are generally available. An example of one vector that may be used is pBIN19.

In a related series of experiments, the cDNAs were cloned into the bacterial vector bluescript. Using this
5 construct, the sequence of the gamma and kappa cDNAs was determined using the methods of Maxam and Gilbert.

Procedures for cloning antibody cDNAs involving PCR techniques or by construction of cDNA libraries followed by ligation of the obtained cDNAs into appropriate vectors
10 are commonplace techniques which are familiar to one of ordinary skill in the art.

b) Hybrid cDNAs encoding the Guy's 13 heavy chain variable region, a part of the gamma chain constant region
15 and a part of an alpha chain constant region.

These constructs were synthesized as described in Ma et al., Eur. J. Immunol., 24:131 (1994) and ligated into the appropriate plant expression vectors as described above. The final construct had the structure: Guy's 13
20 variable region - (IgG1 C_H1) - (IgG1 C_H2) - (IgA C_H2) - (IgA C_H3), referred to as IgG2A heavy chain, and Guy's 13 variable region - (IgG1CH₁) - (IgACH2) - (IgACH3).

c) The Protection Protein and J chain.

The cloned rabbit polyimmunoglobulin receptor (pIgR)
25 cDNA was described by Mostov, Nature, 308:37 (1984) and shown in Figure 8. The protection protein portion was obtained by PCR amplification of a portion of the nucleotide sequence coding for the (pIgR) and ligation into appropriate plant expression vectors as described
30 above. The protection protein portion of the pIgR used in these constructs included the codon for amino acid number 1 to the codon for amino acid number 606. The method to accomplish this construction are well known in the art and the oligonucleotides can be selected using the pIgR
35 nucleic acid sequence.

d) cDNAs encoding aglycosylated derivatives of heavy-chain constant regions.

Mutagenesis procedures were performed either according to Stratagene protocols. In each case (i.e. 5 alpha constant region, or protection protein) the codon for the asparagine utilized as the attachment site for carbohydrates, was changed to a codon for histidine.

2. Production of Transgenic Plants Expressing
10 Therapeutic Antibodies.

Plants and plant cells containing immunoglobulins having a protection protein were produced in the following manner.

a) Transfer of vectors to Agrobacterium
15 tumefaciens.

Plant transformation was accomplished by using Agrobacterium tumefaciens. E. coli DH5 α bearing the recombinant pMON530 plant expression vector were mated with Agrobacterium in the presence of a helper strain 20 (pRK2013) to provide transfer functions. Alternatively, pMON530 plasmid DNA was introduced into Agrobacteria by direct transformation. In this procedure, the Agrobacterium strain was first grown overnight at 28° C in YEP medium. 2 ml of the overnight culture was used to 25 inoculate 50 ml of YEP and was grown to an OD₆₀₀ Of 1.0. The cells were then chilled to 4° C, pelleted by centrifugation and resuspended in 1 ml of ice cold 20 mM CaCl₂. About 1 μ g of DNA was added to aliquots of 0.1 ml of ice cold cells. The cells were then rapidly frozen by 30 immersion in liquid nitrogen or in a dry ice ethanol bath. The cells were thawed by incubation at 37° C for 5 minutes followed by the addition of 1 ml YEP medium. The cells were allowed to incubate for 2-4 hours with gentle shaking. Individual colonies carrying the recombinant 35 vector were isolated by incubation on YEP agar plates containing the appropriate antibiotic.

Agrobacteria containing pMON530 were grown in media containing kanamycin, spectinomycin and chloramphenicol. Small segments of tobacco leaf were then co-cultivated with the *Agrobacterium* for 2 days after which the leaf segments were transferred to plates containing carbenicillin to kill the *Agrobacterium*. Regeneration of transformed leaf cells into whole plants was allowed to proceed in the presence of kanamycin selection until the plants were competent for growth in soil.

10

b) Regeneration of transformed tobacco and petunia plants.

Leaves from greenhouse grown tobacco or petunia plants were sterilized in 20% (by volume) Chlorox bleach, 0.1% sodium dodecyl sulfate at room temperature for 8 minutes. The leaves were then briefly rinsed in 70% ethanol and allowed to dry in sterile Petri plates.

Leaf discs of approximately 0.5 cm diameter were removed with a sterile hole puncher and placed on agar plates containing MS10 medium (MS10 medium per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 0.2 mg naphthalene acetic acid, 2 mg benzylaminopurine, 0.1 mg nicotinic acid, 0.1 mg pyridoxin, 0.1 mg thiamine, 10 g agar, pH 5.7 with KOH).

A 2 ml aliquot of a suspension of *Agrobacterium* in LB (approximately 1×10^8 *Agrobacteria* per ml) was then added to the leaf pieces. All surfaces of the leaf discs were contacted with *Agrobacteria*, excess liquid was poured off the plate, and the discs were co-cultivated with the bacteria for 2 days at room temperature. The discs were then transferred to agar plates containing MS10 medium, 50 $\mu\text{g/ml}$ kanamycin and 250 $\mu\text{g/ml}$ carbenicillin (MS10-KC). Regeneration was allowed to proceed with weekly transfer of discs to fresh MS10-KC plates until regenerating shoots were visible. Shoots were then transferred to agar plates containing MSO-KC medium (MSO-KC per liter: 4.4 g

Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 1 mg nicotinic acid, 1 mg pyridoxin, 0.1 mg thiamine, 50 µg/ml kanamycin and 250 µg/ml carbenicillin, 10 g agar, pH 5.7 with KOH).

- 5 After root formation, plantlets were transferred to soil and grown to maturity.

c) Regeneration of transformed alfalfa plants.

Alfalfa trifoliate were cut from a greenhouse grown
10 plant and sterilized in 20% (v/v) Chlorox bleach, 0.1% sodium dodecyl sulfate at room temperature for 8 minutes. The trifoliate were then briefly rinsed in 70% ethanol and allowed to dry in sterile Petri plates.

Leaf pieces of approximately 1 cm X 4 mm were cut
15 with a sterile scalpel and placed on agar plates containing B5H medium (B5H medium per liter: 3.1 g Gamborg's powdered medium (Sigma #G5893), 500 mg KNO₃, 250 mg MgSO₄ 7H₂O, 30 g sucrose, 500 mg proline, 1 mg 2,4-dichlorophenoxyacetic acid, 100 µg kinetin, 100 mg
20 inositol, 1 mg nicotinic acid, 1 mg pyridoxin, 10 mg thiamine, 10 g agar, 30 ml stock amino acids, pH 5.7 with KOH; stock amino acids consist of 26.6 g L-glutamine, 3.32 g serine, 16.8 mg adenine, 333 mg glutathione per liter and are added after autoclaving when the medium is
25 approximately 50° C).

To the leaf pieces was then added 2 ml of a suspension of Agrobacterium in LB (approximately 1 x 10⁸ Agrobacteria per ml). All surfaces of the leaf were contacted with Agrobacteria, excess liquid was poured off the plate,
30 and the leaves were co-cultivated with the bacteria for 2 days at room temperature. The leaf pieces were then transferred to agar plates containing B5H medium, 25 µg/ml kanamycin and 250 µg/ml carbenicillin (B5H-KC). Regeneration was allowed to proceed with weekly transfer
35 of leaf pieces to fresh B5H-KC plates until somatic embryos were visible. Embryos were then transferred to agar plates containing BI02Y-KC medium (BI02Y-KC per

liter: 25 ml macronutrients, 10 ml micronutrients, 25 ml iron, 1 ml vitamins, 1 ml aminos, 2 g yeast extract, 100 mg myo-inositol, 30 g sucrose, 10 g agar, 25 mg kanamycin, 250 mg carbenicillin, pH 5.9 with KOH; macronutrients
5 consist of 40 g KNO₃, 40 g NH₄NO₃, 13.88 g Ca(NO₃)₂·4H₂O, 1.4 g MgSO₄·7H₂O, 2.6 g KCl, 12 g K₂HPO₄ per liter yielding a 40X stock; vitamins consist of 100 mg thiamine HCl, 500 mg nicotinic acid, 100 mg pyridoxin-HCl per liter yielding a 1000X stock; aminos consists of 2 g per liter glycine
10 yielding a 1000X stock; micronutrients consist of 580 mg MnSO₄·4H₂O, 1550 mg ZnSO₄·7H₂O, 160 mg H₃BO₃, 80 mg KI per liter yielding a 100X stock; iron consists of 1.28 g NaFeEDTA per liter yielding a 40X stock).

After root formation, plantlets were transferred to
15 soil and grown to maturity.

d) Regeneration of Transformed Tomato Plants.

Cotyledons from 7 day old tomato seedlings were sterilized in 20% (v/v) Chlorox bleach, 0.1% sodium
20 dodecyl sulfate at room temperature for 8 minutes. The leaves were then briefly rinsed in 70% ethanol and allowed to dry in sterile Petri plates.

Cotyledon pieces of approximately 0.5 cm diameter were cut with a sterile scalpel and placed on agar plates
25 containing MS4 medium (MS4 medium per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 2 mg zeatin riboside, 5 mg nicotinic acid, 0.5 mg pyridoxin, 0.5 mg thiamine, 1 mM acetosyringone, 10 g agar, pH 5.7 with KOH).

30 To the leaf pieces was then added 2 ml of a suspension of Agrobacterium in LB (approximately 1×10^8 Agrobacteria per ml). All surfaces of the leaf discs were contacted with Agrobacteria, excess liquid was poured off the plate, and the discs were co-cultivated with the
35 bacteria for 2 days at room temperature. The discs were then transferred to agar plates containing MS4 medium minus acetosyringone containing 50 µg/ml kanamycin and 250

μ g/ml carbenicillin (MS4-KC). Regeneration was allowed to proceed with weekly transfer of discs to fresh MS4-KC plates until regenerating shoots were visible. Shoots were then transferred to agar plates containing MSO-KC medium (MSO-KC per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 1 mg nicotinic acid, 1 mg pyridoxin, 10 mg thiamine, 50 μ g/ml kanamycin and 250 μ g/ml carbenicillin, 10 g agar, pH 5.7 with KOH).

After root formation, plantlets were transferred to soil and grown to maturity.

e) Regeneration of Transformed Arabidopsis Plants.

Intact roots derived from *Arabidopsis thaliana* plants grown in sterile culture were first pretreated on callus inducing medium (CIM) for 3 days at 28° C in the dark (CIM medium per liter: 3.1 g Gamborg's powdered medium (Sigma #G5893), 30 g sucrose, 1 mg 2,4-dichlorophenoxyacetic acid, 100 μ g kinetin, 1 mg inositol, 0.1 mg nicotinic acid, 0.1 mg pyridoxin, 0.1 mg thiamine, 8 g agar, pH 5.7 with KOH).

To the intact roots was then added 2 ml of a suspension of *Agrobacterium* in LB (approximately 1×10^8 *Agrobacteria* per ml). All surfaces of the roots were contacted with *Agrobacteria* and excess liquid was poured off the plate. The intact roots were then cut into 5 mm segments and were co-cultivated with the *Agrobacteria* for 2 days at 28° C on CIM plates. The root pieces were then transferred to agar plates containing shoot inducing medium (SIM) containing 50 μ g/ml kanamycin and 250 μ g/ml carbenicillin (SIM medium per liter: 3.1 g Gamborg's powdered medium (Sigma #G5893), 30 g sucrose, 5 mg N⁶-(2-isopentenyl) adenine, 150 μ g indole-3-acetic acid, 1 mg inositol, 0.1 mg nicotinic acid, 0.1 mg pyridoxin, 0.1 mg thiamine, 8 g agar, pH 5.7 with KOH).

Regeneration was allowed to proceed with weekly transfer of root pieces to fresh SIM plates until green

regenerating shoots were visible. Shoots were then transferred to agar plates containing EM medium (MSO-KC per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M6899], 10 g sucrose, 1 mg indole-3-butyric acid 1 mg nicotinic acid, 0.1 mg pyridoxin, 0.1 mg thiamine, 250 µg/ml carbenicillin, 8 g agar, pH 5.7 with KOH).

After root formation, plantlets were transferred to soil and grown to maturity.

10

3. Identification of Transgenic Plants.

Kanamycin resistant transformants expressing individual immunoglobulin chains were identified by ELISA as described. Further analysis of the transformants included evaluation of RNA by Northern blotting and evaluation of immunoglobulin polypeptides by Western blotting, both as described in Maniatis et al.

For each immunoglobulin chain, antigenic material, RNA or protein were detected by the respective assays. Transformants identified as having the highest levels of immunoglobulin chains were used in cross pollination protocols.

4. Assembly of Antibodies by Cross Pollination of Transformants.

Cross pollinations were performed in order to obtain plants co-expressing the various components of the desired antibodies. These crosses yielded alfalfa, tomato, tobacco and *Arabidopsis* plants containing the following assembled components, all of which also contained the Guy's 13 antigen binding domain.

| <u>Type of Antibody</u> | <u>Immunoglobulin Components</u> |
|-------------------------|--|
| 1 | G1 heavy chain, kappa light chain |
| 2 | G2/A heavy chain, kappa light chain |
| 3 | G2/A heavy chain, kappa light chain, |
| 5 | J chain |
| 4 | G1/A heavy chain, kappa light, J chain, protection protein |
| 5 | G1/A heavy chain Kappa light chain |

10 5. Extraction and Evaluation of Guy's 13 Type 1, 2 and 3 & 4 Antibodies From Transgenic Plants.

a) Extraction and enrichment of antibody contained in leaf.

Leaf pieces were chopped into approximately 1 cm² pieces. The pieces were then added to a cold solution of TBS having 10µg/ml leupeptin (1 ml TBS per gram of leaf) contained in a chilled porcelain mortar both at approximately 4° C. Plant liquid was extracted by pulverizing the pieces with a cold pestle using a circular motion and hand pressure. Pulverizing was continued until the pieces became a nearly uniform pulp (approximately 3 minutes of pulverizing). The pulp was centrifuged at 4° C and approximately 50,000 X g to yield a supernatant devoid of solid plant pieces. Alternatively, the pulp was filtered through a plastic mesh with a pore size of approximately 100 microns.

Depending on the titer of antibody contained in the particular plant, the supernatant was either directly suitable for exposure to antigen or required enrichment to a suitable concentration. Yields of IgG1's or IgG/A's in the crude extract were routinely less than 10 µg/ml and averaged approximately 5 µg/ml. For applications of a Guy's 13 antibody to mucosal surfaces, enrichment to a concentration of 1 to 4 mg/ml may be required. As a Type 1, 2 or 3 construct, Guy's 13 antibody required a ten to forty-fold enrichment to yield the desired concentration. This was accomplished either by affinity adsorption

(utilizing either Protein A or Protein G), or by lyophilization to remove water. Size exclusion chromatography was also used for enrichment but required complete fractionation of the crude extract to yield an antibody of the required concentration. By ELISA assay and by polyacrylamide gel electrophoresis, the co-expressed chains assembled into a complex of approximately 180-200 k daltons for types 1 & 2 and approximately 400 k daltons for type 3. Crude extracts were routinely obtained containing approximately of 5-10 $\mu\text{g/ml}$.

A dramatic increase in antibody accumulation was observed when the protection protein was crossed into a plant containing Type 3 antibody yielding a plant containing a Type 4 antibody. By ELISA assay and by polyacrylamide gel electrophoresis, the co-expressed chains assembled into a complex of approximately 470,000 daltons. Crude extracts were routinely obtained containing in excess of 200 $\mu\text{g/ml}$ with an average of approximately 250 $\mu\text{g/ml}$. Therefore, the SIgA construct of the Guy's 13 antibody required minimal enrichment to achieve the target concentration. This enrichment could be accomplished by the techniques described above. Alternatively, it was found that the antibody is readily separated from the majority of plant molecules by a one ultrafiltration step using membrane with a molecular exclusion of 200,000 d.

b. Functionality of the Guy's 13 Type 4 Antibody.

Functional antibody studies were carried out by ELISA. All plants expressing antibody light and heavy chains assembled functional antibody that specifically recognized streptococcal antigen (SA I/II). The levels of binding and titration curves were similar to those of mouse hybridoma cell supernatants. No SA I/II binding was detected with plants expressing only J chain or only protection protein. Likewise, wild-type plants expressing no immunoglobulin showed no detectable levels of binding.

In a similar set of experiments, binding of antibody to immobilized purified streptococcal antigen or native antigen on the bacterial cell surface was detected using an anti-secretory component antiserum. In these assays, only the Type 4 antibody binding was detected. The functional Type 1, 2 or 3 antibodies did not bind the anti-secretory component antiserum. These results confirm that the protection protein was assembled with antibody in the plants expressing Type 4 constructs and in a manner which did not interfere with antigen binding.

6. Expression of Chimeric Immunoglobulins.

The genes encoding the heavy and light chains of a murine monoclonal antibody (mAb Guy's 13) have been cloned and expressed in *Nicotiana tabacum*. Transgenic plants have been regenerated that secrete full-length Guy's 13 antibody. By manipulation of the heavy chain gene sequence, constant region domains from an immunoglobulin alpha heavy chain have been introduced, and plants secreting Guy's 13 mAb with chimeric gamma/alpha heavy chains have also been produced. For each plant antibody, light and heavy chains have been detected by Western blot analysis and the fidelity of assembly confirmed by demonstrating that the antibody is fully functional, by antigen binding studies. Furthermore, the plant antibodies retained the ability to aggregate streptococci, which confirms that the bivalent antigen-binding capacity of the full length antibodies is intact.

30 a. Cloning of heavy and light chain genes

Messenger RNA was purified from the Guy's 13 and a murine IgA (MOPC315) hybridoma cell line, using an acid guanidiniumthiocyanate-phenol-chloroform extraction. Complementary DNA was made using Moloney murine leukemia virus reverse transcriptase (Promega, GB). DNA encoding the gamma and kappa chains of Guy's 13 were amplified by polymerase chain reaction (PCR). The degenerate

oligonucleotides used in the PCR were designed to incorporate a 5' terminal XhoI, and a 3'-terminal EcoRI restriction site in the amplified DNA fragments. Following restriction enzyme digestion, the immunoglobulin chain encoding DNA was ligated into a constitutive plant expression vector (pMON 530), which contains a mouse immunoglobulin leader sequence upstream of the cloning site. The recombinant vector was used to transform *E. coli* (DH5- α , Gibco BRL) and screening was by Southern blotting, using radiolabeled DNA probes derived from the original PCR products. Plasmid DNA was purified from positive transformants and introduced into *Agrobacterium tumefaciens*.

A similar approach was used to construct two forms of a hybrid Guy's 13 heavy chain. The synthetic oligonucleotides shown in Fig. 1 were used in PCR to amplify the regions: (a) Guy's 13 signal sequence to the 3' end of C γ 1 domain (J1-J5), (b) Guy's 13 signal sequence to the 3' end of C γ 2 domain (J1-J2), and (c) 5' end of C α 2 domain to the 3' terminus of DNA from the MOPC 315 hybridoma (J3-J4). The fragments were purified (Geneclean II, Bio 101, La Jolla, CA) and digested with HindIII for 1 h at 37°C. The Guy's 13 fragments were ligated to the MOPC 315 fragment with T4 DNA ligase (Gibco, BRL), at 16°C for 16 h, and an aliquot of the reaction mixture was used as template DNA for a further PCR, using the 5' terminal oligonucleotide for Guy's 13 (J1) and the 3' terminal oligonucleotide for MOPC 315 (J4). Amplified DNA fragments were purified and ligated into the pMON 530 vector as described above. The vector used in this procedure did not have a previously inserted mouse leader sequence, as in this case, the DNA encoding the native Guy's 13 leader sequence was included in the PCR amplification.

35 b. Plant transformation and regeneration

Leaf discs, about 6 mm in diameter, were cut from surface-sterilized tobacco leaves (*Nicotiana tabacum*, var.

xanthii) and incubated overnight at 28°C, with a culture of the recombinant *A. tumefaciens*, containing immunoglobulin cDNA inserts. The discs were transferred to culture plates containing a medium that induces
5 regeneration of shoots, supplemented with kanamycin (200 mg/l) and carbenicillin (500 mg/l). Shoots developing after this stage were excised and transplanted onto a root-inducing medium, supplemented with kanamycin (200 mg/l). Rooted plantlets were transplanted into soil as
10 soon as possible after the appearance of roots. Plants were screened for expression of immunoglobulin chains as described below. Those that expressed heavy chains were crossed with those expressing light chains, by cross-pollination. The resulting seeds were sown in soil and
15 allowed to germinate. Twenty-two transgenic plants were regenerated from transformations with light or heavy chain constructs, as determined by ELISA. Crossing of light and heavy chain-secreting plants resulted in 3/10 F1 progeny plants expressing kappa and gamma chains together, 4/17
20 plants expressing both kappa and the plant G1/A heavy chain and 3/8 plants expressing both kappa and the plant G2/A heavy chain together.

The three different forms of Guy's 13 monoclonal antibody expressed in plants, therefore, all contain the
25 identical light (kappa) chain, but different heavy chains. These will be abbreviated throughout this report as follows (Fig. 1): Guy's 13 IgG1 with original gamma heavy chain, plant G13, Guy's 13 with IgG/IgA hybrid heavy chain consisting of var- $\gamma 1$ - $\gamma 2$ - $\alpha 2$ - $\alpha 3$ domains, plant G2/A. The
30 Guy's 13 hybridoma cell culture supernatant used as a positive control will be abbreviated to Mouse G13. Negative control plants were those that had been transformed with pMON 530 vector containing an insert that encodes an irrelevant mouse protein.

c. Antibody chain detection

Production of either gamma, kappa or the gamma/ alpha chain hybrids was detected by ELISA. Microtiter wells were coated with a goat anti-mouse heavy or light chain-specific IgG (Fisher, USA; Sigma, GB; Nordic Pharmaceuticals, GB) in 150 mM NaCl, 20 mM Tris-HCl (pH 8) (TBS). Blocking was with 5% non-fat dry milk in TBS at 4°C overnight. Plant leaves were homogenized in TBS with leupeptin (10 µg/ml) (Calbiochem, USA). The supernatant was added in serial twofold dilutions to the microtiter plate and incubation was at 4°C overnight. After washing with TBS with 0.05% Tween 20, bound immunoglobulin chains were detected with the appropriate goat anti-mouse heavy or light chain-specific antibody, conjugated with horseradish peroxidase (Fisher; Sigma; Nordic Pharmaceuticals), for 2 h at 37°C. Detection was with 2,2'-azino-di-(3-ethyl-benzthiazoline-sulfonate) (Boehringer, FRG).

A similar assay was used to determine the concentrations of the murine and plant Guy's 13 antibodies. These were compared with a mouse IgG1 mAb (MOPC 21), and a mouse IgA mAb (TEPC 21) used at known concentrations (Sigma). ELISA plates were coated with an anti-mouse kappa antiserum. After blocking, bound antibody was detected with horseradish peroxidase-labeled anti-mouse gamma or alpha antiserum. Antibody concentration was determined by comparison of binding curves for each antibody.

ELISA was also used to detect the binding function of the assembled antibody. Binding to SA I/II was detected using microtiter plates that had been coated with purified SA I/II at an optimized concentration of 2 µg/ml. The ELISA procedure was as described above. The ability to bind *S. mutans* or *E. coli* cells was detected using intact cells (strains Guy's c, *S. mutans* and DH5-α, *E. coli*) that had been grown to stationary phase, for 18 h at 37°C and fixed in 10% formalin. All the antibody solutions were adjusted to an initial concentration of 1.5 µg/ml and used

in serial twofold dilutions. Extracts from plants expressing wither Guy's 13 heavy or light chain singly were also included in these assays, to determine if the single immunoglobulin chains exhibited any antigen-binding activity. Antibodies bound to either cells or purified SA I/II were detected using a horseradish peroxidase-conjugated goat anti-mouse light or heavy chain antiserum (Nordic Pharmaceuticals). The results are expressed as mean \pm standard deviation of duplicate results from three separate assays.

Competition ELISA was performed on microtiter plates coated with purified SA I/II as above. The plates were incubated with plant extracts of Guy's 13 hybridoma supernatant at 1.5 μ g/ml and serial twofold dilutions at 37°C for 1 h and 4°C overnight. After washing, 125 I-labeled mouse Guy's 13 was added and left to incubate for 2 h at 37°C. The plates were washed again and the bound radioactivity was counted in a gamma counter (Hydragamma 16, Innotec, GB). The results are expressed as % inhibition of labeled mouse Guy's 13 binding, in which 100% is the radioactive count from wells to which no blocking solution had been added.

d. Western blot analysis

Aliquots of 10 μ l of leaf homogenates were boiled with 75 mM Tris-HCl (pH 6.8), 2% SDS, under reducing and non-reducing conditions. SDS-PAGE in 10% acrylamide was performed, and the gels were blotted onto nitrocellulose. The blots were incubated for 16 h in TBS with 0.05% Tween 20 and 1% non-fat dry milk, followed by goat anti-mouse IgG1, kappa (Nordic Pharmaceuticals) or alpha chain-specific antisera (Sigma), and incubated for 2 h at 37°C. After washing, the second-layer antibody, an alkaline phosphatase-conjugated rabbit anti-goat IgG (Sigma) was applied for 2 hours at 37°C. Antibody binding was detected by incubation with 300 μ g/ml nitroblue

tetrazolium and 15p µg/ml 5-bromo-4-chloro-3-idolyl phosphate (Promega).

e. DNA sequencing

5 The DNA sequence of each cloned immunoglobulin gene insert confirmed that no mutations had occurred during PCR amplification or the cloning procedures. The introduction of the HindIII site in the λ/γ hybrid heavy chains resulted in the predicted addition of the leucine residue
10 between the Cγ2 and Cα2 domains in Plant G2/A and leucine-lysine between the Cγ1 and Cα2 domains in Plant G1/A. The additional Cγ2 domain in the Plant G2/A construct is predicted to increase the length of the heavy chain by 141 amino acid residues (approximately 12000 Da). The plant
15 G1/A heavy chain is predicted to be slightly larger than the native Guy's 13 heavy chain, by 33 amino acids, approximately 3000 Da.

Plasmid DNA that was purified from positive transformants in *E. coli* was sequenced. The immunoglobulin gene
20 inserts were excised and sub-cloned into Bluescript (Stratagene, USA). The DNA sequence was determined by a di-deoxy termination procedure (Sequenase, USB, USA).

f. Expression of assembled antibody

25 Western blot analysis on extracts from three representative F1 progeny plants was performed and reported in Figure 2 of Ma et al., Eur. J. Immunol., 24:131-138 (1994). Samples run under reducing conditions demonstrate the presence of light (kappa) chain at approximately 25
30 Kd, in the mouse Guy's 13, as well as in the three transgenic plants, but not in the control plant. Guy's 13 heavy (gamma) chain was also detected in plant G13 at approximately 57 Kd, but not in the control plant extract. A single protein species was detected, unlike the
35 hybridoma producing the Guy's 13 antibody cell culture supernatant, in which a two protein species was a consistent finding. The difference in the molecular size

of the mouse heavy chains is probably due to glycosylation differences, and the result suggests that in plants the two heavy chains may be glycosylated in the same way.

The heavy chains of plant G1/A and G2/A were detected
5 with an anti-alpha chain antiserum. Compared with the mouse Guy's 13 heavy chain, (approximately 57 Kd), the heavy chain of plant G1/A has a slightly higher relative molecular mass (approximately 60 Kd) and the plant G2/A heavy chain is much larger (approximately 70 Kd). This is
10 consistent with the molecular weights predicted by sequence analysis. Several other protein species were detected in the transgenic plant extracts. These are likely to be proteolytic fragments of either light/heavy chain complexes, or of the heavy chain, as no bands were
15 detected in the extract from the control transgenic plant. The anti-alpha chain antiserum did not cross-react with the mouse Guy's 13, which only contains gamma chain domains.

Samples were also run under nonreducing conditions to
20 confirm the assembly of heavy and light chains into an immunoglobulin molecule and reported in Figure 3 of Ma et al., Eur. J. Immunol., 24:131-138 (1994). Detection was with a labeled anti-kappa antiserum, and all three transgenic plants had assembled immunoglobulin at the
25 correct M_r of above 150 Kd for full-length antibody. The plant G13 antibody has the same M_r as the mouse G13, but the plant G2/A and plant G1/A antibodies have higher M_r as predicted. A number of smaller proteolytic fragments were also detected, which is consistent with previous findings
30 and the fact that a number of proteases are released by plants during the antibody extraction procedure. That these are antibody fragments, is confirmed by the absence of any detectable bands in the control plant extract.

35 g. Antigen binding

Ten plants which were producing immunoglobulin were made in total, and the concentration of immunoglobulin in

plant extracts varied between 1 and 10 $\mu\text{g/ml}$ (mean 4.5 $\mu\text{g/ml}$). For the murine antibody and the representative plants used in this study, the concentrations estimated by ELISA were: mouse IgG-15.4 $\mu\text{g/ml}$, plant IgG-7.7 $\mu\text{g/ml}$,
5 plant G1/A-1.5 $\mu\text{g/ml}$ and plant G2/A-2.1 $\mu\text{g/ml}$. The concentrations determined for plant antibodies containing hybrid heavy chains are possibly underestimated, as they do not carry all of the constant region determinants, as compared with the standard mAb IgA used.

10 Titration curves for extracts from the three representative transgenic plants binding to SA I/II were generated and reported in Figure 4 of Ma et al., Eur. J. Immunol., 24:131-138 (1994). Specific antibody was detectable in all three transgenic plant extracts, and the
15 titration curves were similar to that of the murine hybridoma cell culture supernatant, used at the same concentration. The binding of the plant G1/A antibody appeared to be slightly lower than the other antibodies, although the titration curve followed a similar pattern.
20 No SA I/II binding activity was detected in the negative control plant nor did extracts from plants individually expressing light or heavy chains have binding activity towards purified SA I/II. These findings demonstrate that the transgenic plants expressing both light and heavy
25 chains have assembled the antibody molecule correctly to form a functional antigen binding site and that single light or heavy chains are not capable of binding the antigen.

The plant antibodies also recognized native antigen
30 on the surface of streptococcal cells as shown in Figure 5 of Ma et al., Eur. J. Immunol., 24:131-138 (1994) (*S. mutans* serotype c), which further confirms the integrity of the antigen-binding site in the plant antibodies. There were no significant differences between the binding
35 of the different antibodies. Neither extracts from control plants, nor plants expressing only heavy or light chains showed any binding to *S. mutans* cells. There was

no binding to *E coli* cells by any of the plant extracts, at concentrations of 1.0 and 0.5 µg/ml.

The plant antibodies competed with the original mouse Guy's 13 mAb for binding to SA I/II. Up to 85%
5 inhibition of ¹²⁵I-labeled mouse Guy's 13 mAb binding to SA I/II was demonstrated using the plant antibodies as shown in Figure 6 of Ma et al., Eur. J. Immunol., 24:131-138 (1994). As before, the inhibition titration curves of the plant antibodies were similar to each other, and
10 comparable to that of the mouse Guy's 13, whereas the control plant extract gave no inhibition.

h. Aggregation of *S. mutans*

The action of the immunoglobulin produced in plants
15 having the Guy's 13 antigen binding region on bacteria was determined and reported in Figure 7 of Ma et al., Eur. J. Immunol., 24:131-138 (1994). Plant extracts were sterilized by filtration through a 0.22 µm pore size filter and diluted tenfold with Todd Hewitt broth. The
20 samples were inoculated with 0.05 vol of an overnight *S. mutans* culture and incubated at 37°C overnight. The samples were Gram stained and examined under oil immersion microscopy. *S. mutans* grown in the presence of mouse Guy's 13, plant Guy's 13, plant G1/A or plant G2/A became
25 aggregated and cell clumping was evident. However, the control plant extract had no effect on *S. mutans* growth. None of the plant mAb appeared to affect *S. mutans* rate of growth, as determined by culture of viable organisms at 8, 12 and 16 h. This result demonstrates not only that the
30 plant antibodies have correctly assembled antigen-binding regions, but also that the antibody molecules bind antigen bivalently.

Example 7. Production of Immunoglobulins Containing Protection Proteins

Four transgenic *Nicotiana tabacum* plants were generated to express (1) a murine monoclonal immunoglobulin kappa chain having the antigen binding site of the Guy's 13 light chain, (2) a hybrid IgA/G murine immunoglobulin heavy chain containing C γ and C α chain domains and the antigen binding site of the Guy's 13 heavy chain, (3) a murine J chain and (4) protection protein comprised of amino acids 1-606 of rabbit polyimmunoglobulin receptor and did not contain amino acids 627-675 of the rabbit polyimmunoglobulin receptor. See, Example 1. Successive sexual crosses between these plants resulted in simultaneous expression of all four protein chains in the progeny plants. In some cases, back crossing was used to produce homozygous plants. The four recombinant polypeptides were assembled into a functional, high molecular weight immunoglobulin containing a protection protein of approximately 470,000 Kd. The assembly of the protection protein with the immunoglobulin was dependent on the presence of a J chain, as no association of the protection protein was detected when plants expressing antibody alone were crossed with those expressing the protection protein. Microscopic evaluation of plants expressing the immunoglobulins containing the protection protein demonstrated co-incident expression of protection protein and immunoglobulin heavy chains in single cells. Single cells are able to produce immunoglobulin having a protection protein in transgenic plants, whereas two cells are required for natural production of secretory immunoglobulin in mammals. The results demonstrate that sexual crossing of transgenic plants expressing recombinant sub-units is suitable for large scale production of immunoglobulin containing a protection protein for passive immunotherapy, as well as for expressing other complex protein molecules.

The immunoglobulin which contains the protection protein has the heavy and light chain antigen binding domains from the Guy's 13 monoclonal antibody that specifically recognize the cell surface adhesion molecule SA 1/11 of an oral streptococcus as shown by Smith, R. & Lehner, T. *Oral Microbiol. Immunol.* 4, 153-158 (1989). Transgenic immunoglobulin of this type containing only heavy and light chains has been generated in *Nicotiana tabacum* plants as described in Example 6. A mouse J chain construct containing the coding length cDNA was amplified using synthetic oligonucleotide primers corresponding to the N terminus MKTHLL and the C terminus SCYPD of mouse J chain as described by Matsuuchi, L., Cann, G. M. & Koshland, M.E. *PNAS* 83, 456-460 (1986). This amplified nucleotide sequence was ligated into a constitutive plant expression vector, pMON 530, that includes the 35S promoter from Cauliflower Mosaic Virus and has been described by Rogers, S. G., Klee, H. J., Horsch, R. B. & Fraley, R. T. *Meth. Enzymol.* 153, 253-276 (1987). Tobacco leaf tissue was transformed using agrobacterium containing the recombinant plasmid as described in the previous Examples. Regenerated plants were screened for the production of messenger RNA encoding J chain and positive transformants were self fertilized in order to generate homozygous progeny. The J chain expressing plants were crossed initially with those expressing the chimeric immunoglobulin heavy chain and kappa chain. Western blot analysis of the plant extract from plants expressing the chimeric immunoglobulin heavy chain with anti-kappa antiserum under non-reducing conditions, revealed a protein species of approximately 210 Kd, which is consistent with the presence of the extra constant region domains present in the chimeric immunoglobulin heavy chain, as compared with the original IgG1 antibody. The progeny from the cross between the plant expressing the immunoglobulin and a J chain plant resulted in the appearance of a major immunoglobulin band at approximately

twice the relative molecular mass of approximately 400 Kd, demonstrating that assembly of the 3 polypeptides had occurred to form dimeric immunoglobulin (dlgA/G).

The protection protein construct consisted of a
5 coding length cDNA amplified using synthetic
oligonucleotide primers corresponding to the N terminus
MALFLL and AVQSAE at amino acids 601-606 of the C terminus
of rabbit polyimmunoglobulin receptor. The nucleotide
sequence of the rabbit polyimmunoglobulin receptor was
10 reported by Mostov, K. E., Friedlander, M. & Blobel, G.
Nature 308, 37-43 (1984). The protection protein was
generated in transgenic plants as described above and
positive transformants expressing the protection protein
were identified by Western blot analysis.

15 Plants expressing J chain assembled with the
immunoglobulin having the IgA/G heavy chains to form
dimers were then crossed with a homozygous plant
expressing the protection protein. The progeny plants
expressing the immunoglobulin having the protection
20 protein contained a higher molecular weight protein
species at approximately 470 Kd as determined by Western
blot analysis under non-reducing conditions. This
molecular size was consistent with that expected for an
immunoglobulin containing a protection protein. This high
25 molecular weight protein contained the protection protein
as confirmed by Western blotting, using antiserum that
specifically recognized the protection protein. The plant
extracts also contained a protein species of approximately
400 Kd corresponding to the dimers of IgA/G and a protein
30 species of approximately 210 Kd corresponding to the
immunoglobulin with the chimeric heavy chain, but these
were only detected by anti-kappa antiserum and not the
anti-protection protein antiserum. In the transgenic
plant producing the protection protein alone, there was no
35 evidence that the protection protein assembled with
endogenous plant proteins or formed multimers, as no high
molecular weight proteins were detected in Western

blotting under non-reducing conditions. Western blot analysis demonstrated that extracts from the plants expressing immunoglobulin heavy chain (IgA/G, dimeric IgA/G and the immunoglobulin containing a protection protein), but not the plants containing only the protection protein or J chain or wild-type plants, contained identical immunoglobulin derived heavy and light chains. Furthermore, only the plants containing protection proteins and the plants containing the IgG/A immunoglobulin having the protection protein expressed proteins that were recognized by the antiserum that specifically recognized the protection protein. No cross reacting proteins were detected in extracts from the wildtype control plant.

15 In mammals, the assembly of secretory component with the immunoglobulin requires the presence of J chain as described by Brandtzaeg, P. & Prydz, H. *Nature* 311, 71-73 (1984). Plants expressing immunoglobulins containing a chimeric heavy chain (IgA/G) were crossed with plants expressing protection protein. None of the 10 resulting progeny that expressed immunoglobulin and the protection protein without J chain produced assembled complexes as compared with the 10/10 plants that co-expressed J chain dimerized immunoglobulin and the protection protein without J chain, which assembled the M_r 470 Kd immunoglobulin containing the protection protein. This confirms that J chain is required for the protection protein association with immunoglobulin as found in mammals. Only the approximately 210 Kd monomeric form of the immunoglobulin was recognized by anti-kappa antiserum, and the antisera that specifically bound the protection protein, recognized free protection protein, but no immunoglobulin heavy or light chains proteins.

Functional studies were carried out using the immunoglobulin produced in the 5 plant constructs using ELISA. All plants expressing immunoglobulin light and heavy chains, assembled functional immunoglobulin that

specifically recognized streptococcal antigen (SA I/II). The levels of binding and titration curves were similar to those of the native mouse hybridoma cell supernatant. No SA I/II binding was detected in plants expressing only J chain or only protection protein or in wildtype plants. Binding of the immunoglobulins to immobilized purified streptococcal antigen or to native antigen on the bacterial cell surface was also detected using the antiserum which specifically binds the protection protein. In these assays, the binding of the immunoglobulin containing the protection protein to the streptococcal antigen was specifically detected. These results confirmed that the protection protein was assembled with the immunoglobulin to produce an immunoglobulin containing a protection protein in a manner which did not interfere with antigen binding.

The assembly of heavy and light chains into functional immunoglobulin molecules in plants is very efficient as shown by Hiatt, A. C., Cafferkey, R. & Bowdish, K. *Nature* 342, 76-78 (1989). A signal peptide must be present on both heavy and light chain constructs to direct the recombinant proteins to the endoplasmic reticulum antibody for assembly to take place in plants as was previously shown by Hiatt, A. C., Cafferkey, R. & Bowdish, K. *Nature* 342, 76-78 (1989). This study has demonstrated the fidelity of immunoglobulin assembly which includes dimerization of monomeric antibody by J chain in the transgenic plants. These results demonstrated that in plants the dimeric immunoglobulin population represents a major proportion (approx. 57%) of the total antibody. These results also demonstrate the production of an assembled immunoglobulin containing a protection protein which binds the corresponding antigen as well as the parent murine monoclonal antibody, which makes up a major proportion of the total antibody when the protection protein is incorporated (approximately 45%).

Co-expression of dimeric immunoglobulin with the protection protein in plants has led to assembly of a functional immunoglobulin containing a protection protein. All four transgenes for this complex protein were introduced into plants with the identical pMON530 expression cassette and native leader sequences. This vector contains a promoter sequence derived from the 35S transcript of the cauliflower mosaic virus which directs expression of transgenes in a variety of cell types of most plant organs as has been described by Benfey, P. N. & Chua, N-H. *Science* 250, 959-966 (1990); and Barnes, W. M. *PNAS* 87, 9183-9187 (1990). Directing expression of all four transgenes with the same promoter maximized the likelihood of coincidental expression in a common plant cell. Microscopic observation of plants expressing an immunoglobulin containing a protection protein revealed that many cell types of the leaves contain the individual protein components that make up the immunoglobulin. These proteins accumulated at highest concentration in bundle sheath cells and were confined by the cell walls of these and other cells, but were not found in intercellular spaces. Restriction of the largest immunoglobulin components, the protection protein and the chimeric immunoglobulin heavy chain, within the confines of a protoplasmic or apoplasmic compartment of individual cells would constrain the assembly of the secretory immunoglobulin to those cells in which all the component molecules are synthesized. The subcellular site(s) and mechanism of assembly remain to be determined, assembly of IgG heterotetramers in plants requires targeting of both proteins to the endomembrane system as has been previously shown by Hiatt, A. C., Cafferkey, R. & Bowdish, K. *Nature* 342, 76-78 (1989); and Hein, M. B., Tang, Y., McLeod, D. A., Janda, K. D. & Matt, A. C. *Biotechnol Prog.* 7, 455-461 (1991).

In addition, we have demonstrated that a protection protein derived from mature secretory component devoid of

signals for membrane integration, transcytosis or subsequent proteolysis can be assembled with chimeric immunoglobulin heavy chain containing immunoglobulin gamma and alpha protein domains. These results demonstrate that the inherent functions of IgG constant regions (protein A binding, complement fixation, Fc receptor activity) may be maintained in a dimeric immunoglobulin, capable of binding to a protective protein. These additional capabilities may be employed to enhance the function of an immunoglobulin used for passive immunotherapy and the development of plants capable of generating a functional immunoglobulin containing a protection protein will have significant implications in passive immunotherapy. The level of expression of the immunoglobulin containing a protection protein is high and the production can be scaled up to agricultural proportions, to allow economical production of monoclonal antibodies.

Methods

The following methods were used to prepare and analyze the Immunoglobulin of this Example.

- i) Antibody assembly in transgenic *Nicotiana tabacum*.
Leaf segments were homogenized in 150mM NaCl 20mM Tris-HCl (pH8) (TBS), with leupeptin (10µg/ml). The extracts were boiled for 3 minutes, in 75mM Tris-HCl (pH6.8), 2% SDS, under non-reducing conditions and SDS-PAGE in 4% acrylamide was performed. The gels were blotted onto nitrocellulose. The blots were incubated for 2 hrs in TBS with 0.05% Tween 20 and 1% non-fat dry milk, followed by the appropriate antiserum and incubated for 2 hrs at 37°C. After washing, the second layer alkaline phosphatase conjugated antibody was applied for 2 hrs at 37°C. Antibody binding was detected by incubation with 300mg/ml nitroblue tetrazolium and 150mg/ml 5-bromo-4-chloro 3-indolyl phosphate.

These extracts were analyzed using western analysis to determine whether the immunoglobulins were assembled into immunoglobulin molecules by analyzing Western blots of plant extracts prepared under non-reducing conditions, were with anti-kappa antiserum (Bradsure, UK) and an antiserum which specifically recognizes protection protein. The immunoglobulins produced in the plants were compared to the monoclonal IgG1 Guys 13 immunoglobulin described by Smith, R. & Lehner, T. *Oral Microbiol. Immunol.* 4, 153-158 (1989).

ii) Western Analysis.

Western analysis was performed on each of the plant extracts prepared under reducing conditions to identify individual protein components of the immunoglobulin. Samples of the various plant extracts were prepared as described previously, but with the addition of 5% β -mercaptoethanol. SDS-PAGE in 10% acrylamide was performed and the protein in the gels transferred to nitrocellulose. Individual proteins were detected using anti-mouse γ 1 heavy chain (Sigma, UK); anti-mouse kappa chain (Bradsure, UK); or an antiserum that specifically recognized the protection protein, followed by the appropriate alkaline phosphatase conjugated antibody.

iii) Western Analysis to Show Production of Immunoglobulin Having a Protection Protein

Western analysis of transgenic plant extract was performed as described in ii) above. The plant extracts from plants expressing the immunoglobulin containing the protection protein were subjected to SDS-PAGE under both non-reducing and reducing conditions and the proteins transferred to nitrocellulose. The immunoglobulin components were detected with an anti-kappa antiserum or with a sheep antiserum which specifically recognized the protection protein followed by an appropriate alkaline phosphatase labeled 2° antibody.

iv) Expression of Antigen-Specific Immunoglobulin Containing a Protection Protein in transgenic *Nicotiana tabacum*.

To demonstrate that the plants were producing
5 antigen-specific immunoglobulin, plant extract binding to
purified streptococcal antigen (SA) I/II, detected with
horseradish peroxidase labeled anti-kappa chain antiserum
was determined. The presence of a protection protein in
the antigen-specific immunoglobulin was demonstrated by
10 plant extract binding to purified streptococcal antigen
I/II and streptococcal cells detected with a sheep
antiserum immunospecific for a protection protein,
followed by alkaline phosphatase labeled donkey anti-sheep
antiserum. These tests for antigen-specific
15 immunoglobulin were carried out in microtitre plates that
were coated with purified SA I/II (2µg/ml) in TBS, or log
phase growth *Strep. mutans* (NCTC 10449), in bicarbonate
buffer (pH 9.8). Blocking was with 5% non-fat dry milk in
TBS at room temperature for 2 hours. Plant leaves were
20 homogenized in TBS with 10µg/ml leupeptin (Calbiochem,
USA). Mouse Guy's 13 hybridoma cell culture supernatant
(IgG) was used as a positive control. The supernatants
were added in serial two-fold dilutions to the microtitre
plate and incubation was at room temperature for 2 hours.
25 After washing with TBS with 0.05% Tween 20, bound
immunoglobulin chains were detected with either a goat
anti-mouse light chain specific antibody, conjugated with
horseradish peroxidase (Nordic Pharmaceuticals, UK), or a
sheep anti-SC antiserum, followed by an alkaline
30 phosphatase labeled donkey anti-sheep antibody for 2 hours
at room temperature. Detection was with 2,2'-azino-di-[3-
ethyl-benzthiazolin-sulphonate (Boehringer, W. Germany)
for HRPO conjugated antibody or disodium p-nitrophenyl
phosphate (Sigma, UK) for alkaline phosphatase conjugated
35 antibody.

v) Localization of Immunoglobulin Components in Plants

Photomicrographs of transgenic plants expressing immunoglobulins containing protection proteins and control *Nicotiana tabacum* leaf were prepared using immunogold
5 detection of murine alpha chain. Briefly, leaf blades were cut into 2mm x 10mm segments and fixed in 3% (w/v) paraformaldehyde, 0.5% (w/v) glutaraldehyde, 5% (w/v) sucrose in 100mM sodium phosphate (pH 7.4). After dehydration in anhydrous ethanol, leaf segments were
10 infiltrated with xylene, embedded in paraffin and cut into 3mm sections and mounted on glass slides for immunochemical staining. The leaf sections were incubated with primary antibodies, affinity purified rabbit anti-mouse alpha chain (which reacts with the A/G hybrid heavy
15 chain) or sheep anti-rabbit SC, and then with secondary antibody; goat anti-rabbit-10nm gold or rabbit anti-sheep-10nm gold. The immunogold signal was intensified by silver enhancement. The plants were visualized using both Phase contrast and bright field microscopy on the same
20 leaf cross section. Immunolocalization of the protection protein on serial sections was used to show the same cellular localization for heavy chain as immunoglobulin. The analysis was carried out on the following cells and cell compartments:

25 spongy mesophyll cells, epidermal cells, intercellular spaces, palisade parenchyma cells, and vascular bundles.

Further analysis of the exact localization of immunoglobulin components was carried out by analyzing serial sections of *Nicotiana tabacum* vascular bundle and
30 control *Nicotiana tabacum* vascular bundle with immunogold detection for each of the components of the immunoglobulin. Serial sections of a transgenic plant leaves from plants expressing secretory immunoglobulin were incubated with an antibody that specifically
35 recognizes the protection protein or with anti-IgA antibody followed by the appropriate gold-labeled secondary antibody. A control leaf section from a

transgenic plant that did not contain any immunoglobulin coding sequences was also incubated with anti-IgA antibody, followed by gold-labeled goat anti-rabbit antiserum, or with the gold-labeled secondary antibodies alone and confirmed the specificity of staining. Both Phase contrast illumination of a minor vascular bundle and Bright field illumination of the same field were used to show immunogold localization of the protection protein. Bright field illumination of a serial leaf cross section of the vascular bundle demonstrated the same immunogold localization of the immunoglobulin heavy chain as was shown for the protection protein.

Example 8. Production of a Useful Plant Extract
15 Containing Immunoglobulins Having a Protection Protein

Plant pieces (either leaf, stem, flower, root, or combinations) from plants producing immunoglobulins containing a protection protein were mixed with homogenization buffer (2 milliliter buffer per gram of plant material; homogenization buffer: 150 mM NaCl, 20 mM Tris-Cl, pH 7.5), homogenized into a pulp using a Waring blender and centrifuged at 10,000 X g to remove debris. The supernatant was then extracted with an equal volume of HPLC-grade ethyl acetate by shaking at room temperature, followed by centrifugation at 10,000 X g. The aqueous phase was transferred to another container, remaining ethyl acetate was removed from the aqueous phase by placing the solution under vacuum. The resulting crude extract consistently contained 100 μ g immunoglobulin having a protection protein per ml. This method is useful for any plant containing an immunoglobulin having a protection protein.

A number of methods for homogenization have been used including a mortar and pestle or a Polytron and can be performed either in the cold or at room temperature.

The extract may be further purified by delipidation, by extraction with hexane or other organic solvents.

Delipidation is not essential for deriving a useful product from the plant extract but is advantageous in cases where the final product is a purified immunoglobulin having a protection protein. In many instances the crude
5 extract will contain a sufficiently high quantity of immunoglobulin having a protection protein (i.e. 100 $\mu\text{g/mL}$) to be useful without any further purification or enrichment. For an oral application, the extract would be mixed with commonly used flavorings and stabilizers. For
10 a dental application, the extract would in addition be mixed with a gelling reagent to maintain contact of the extract with teeth. For a gastric application, the flavored extract could be swallowed directly.

15 Example 9. Stability of an Immunoglobulin Containing a Protection Protein.

Two sets of crude plant extracts were prepared as described above. The first extract was derived from a plant expressing an IgG1 antibody and the second extract
20 was derived from a plant expressing an immunoglobulin containing a protection protein. Crude plant extracts of this type from plants are known to contain a variety of proteolytic enzymes. Prolonged incubation of extracts at room temperature or at 37° C therefore constitutes a
25 proteolytic digestion.

Using ELISA the quantity of gamma-kappa complexes in the two extracts was determined as a function of time at both room temperature and 37° C. In these assays, an anti-kappa chain antibody was used to coat the plate
30 followed by incubation with the plant extract at 37° C for 1 hour. An anti-gamma chain antibody conjugated to HRPO was used for detection of immunoglobulin derived from the plant. The quantity of immunoglobulin having a protection protein contained in the extract immediately after the
35 extract was prepared was taken to be 100%. After 3 hours at room temperature, the IgG1 contained 40% and the immunoglobulin containing the protection protein contained

>95%. After 6 hours, the remaining IgG1 antibody was 20% and the immunoglobulin containing the protection protein abundance was still >95%. After 12 hours, there was no detectable IgG1 whereas ~90% of the immunoglobulin
5 containing the protection protein remained. A significant decrease (to ~70%) in the abundance of protected antibody was not observed until 48 hours after the extract was prepared.

10 Example 10. Eukaryotic Tetra-transgenic Cells Expressing Immunoglobulins Containing Protection Protein.

The four chains comprising the immunoglobulin containing a protection protein can also be expressed in other cell types either in in vitro (cell cultures) or in
15 vivo (transgenic animals). See, Manipulating the Mouse Embryo; A Laboratory Manual, B. Hogan et al., Cold Spring Harbor Laboratory (1986). In the case of transgenic animals, purified preparations of appropriate vector DNAs are adjusted to a final concentration of 2 ng/ μ l in 10 mM
20 Tris, 0.2 mM EDTA, pH 7.4. Pronuclear injections are performed using zygotes prepared from inbred animals. Injected eggs are then transferred to pseudopregnant females using standard techniques. Live born animals are then screened for the presence of transgenes using any of
25 a number of commonly used techniques such as PCR and ELISA. Members of the pedigree expressing different components of the immunoglobulin containing the protection protein are then mated to produce multi-transgene animals. Progeny from these crosses are then screened to identify
30 those that express all four chains. Depending on the type of vector used for zygotic injections various cell types can be identified in the transgenic animals which assemble the complete immunoglobulin containing a protection protein. These vector DNAs can consist of specific
35 promoter elements which allow transcription of the transgene in particular cell types or tissues. Each vector could express a single component of the protected

antibody (IgG/A, J chain, protection protein, or kappa chain) or could potentially express more than one component. In this instance, the vector would contain an appropriate number of promoter regions and restriction sites to allow for transcription of each transgene.

Expression of all four chains in a cell culture system can be achieved using a DNA vector from which each component can be individually promoted. This would require four expression cassettes (containing promoter, multiple cloning site, and polyadenylation region) on the same vector DNA. Alternatively, individual cell lines can be sequentially transfected with individual vectors expressing single chains so long as each vector confers a selective resistance onto the cell line.

Commonly available vectors, such as pMAMneo (Clontech) can be adapted either for multiple expression or as a series of vectors expressing distinct selectable markers.

Transfection of any eukaryotic cells, such as fibroblasts, is done by conventional techniques. Briefly, cells are split 1:20 the day before transfection and are transfected at approximately 30% confluency using 125 mM CaCl₂, 140 mM NaCl, 25 mM Hepes, 0.75 mM NaHPO₄, pH 7.05, and 5 µg DNA / 10 cm dish. After 16 hours of DNA incubation, cells are shocked by 10% dimethyl sulfoxide for 3 minutes. Forty eight hours after transfection, cells are subjected to selection by growth in the appropriate medium containing an antibiotic or other cytotoxic reagent.

The resulting cells produce all the components for the immunoglobulin containing the protection protein. These components are properly assembled to produce a functional immunoglobulin containing a protection protein.

Example 11. Engineering A Protection Protein Fused to A Portion of the Cytoplasmic Domain of the Rabbit Polyimmunoglobulin Receptor.

The construction of DNA segments encoding a
5 protection protein fused to a segment encoding a segment
of the cytoplasmic domain of the rabbit polyimmunoglobulin
receptor is produced as follows. Protection protein cDNA
encoding from the first amino acid of the signal sequence
(MET₁₈) to GLU₆₀₆ is ligated into any plant expression
10 vector, such as the pMON530 vector (digested with Bgl II
and Xho I) as a Bgl II - Xho I fragment. This protection
protein derivative is obtained by PCR amplification using
the appropriate oligonucleotide primers containing either
a Bgl II or Xho I recognition sequence which are also
15 complementary to DNA encoding residues -18 to -13 and
residues 601 to 606 of the rabbit polyimmunoglobulin
receptor respectively. The same procedure is performed in
order to obtain a protection protein cDNA encoding from
MET₁₈ to ALA₆₂₈ except that the oligonucleotide containing
20 an Xho site is also complementary to the protection
protein cDNA encoding residues 623 to 628.

The cDNA encoding the rabbit polyimmunoglobulin
receptor cytoplasmic domain fragment is obtained, also by
PCR amplification, as a Xho I fragment. The
25 oligonucleotides employed are complementary to DNA
encoding from ARG₆₅₃ to ALA₇₅₅ both containing Xho I
recognition sequences. This fragment is then ligated into
the pMON530 vectors which contain the either of the
protection protein cDNAs described above. The appropriate
30 orientation of the cytoplasmic domain cDNA is determined
by restriction digestions and by sequence analysis of
plasmids obtained from transformed bacterial colonies.

The oligonucleotides employed for PCR amplification
contain the appropriate number of nucleotides to ensure
35 that the resulting cDNAs are in frame and capable of being
translated as a continuous fusion protein containing both
protection protein and cytoplasmic domain.

The resulting constructs in the appropriate orientation encode a protection protein fused directly to the polyimmunoglobulin receptor cytoplasmic domain with no functional transmembrane segment, operably linked to a DNA
5 segment (promoter) enabling expression in a plant cell. The constructs encode two additional amino acids (SER - TRP) which are derived from introduction of the Xho I restriction site and which serve as a linker between the protection protein and the cytoplasmic domain.

10 These vectors are then used to transform *Agrobacterium* as previously described which in turn is used to transform plant cells. The same techniques described in the above Examples are used to produce a plant expressing this protein as part of an
15 immunoglobulin.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: ANDREW C. HIATT, JULIAN
 K.-C. MA, THOMAS LEHNER

 (ii) TITLE OF INVENTION: IMMUNOGLOBULINS CONTAINING PROTECTION
 PROTEINS IN PLANTS AND THEIR USES

10 (iii) NUMBER OF SEQUENCES: 19

 (iv) CORRESPONDENCE ADDRESS:

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 (v) COMPUTER READABLE FORM:

25 (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
 storage
 (B) COMPUTER: IBM Compatible
 (C) OPERATING SYSTEM: IBM P.C. DOS 5.0
30 (D) SOFTWARE: Word Perfect 5.1

 (vi) CURRENT APPLICATION DATA:

 (A) APPLICATION NUMBER: TO BE ASSIGNED
 (B) FILING DATE:
35 (C) CLASSIFICATION:

 (vii) PRIOR APPLICATION DATA:

40 Prior applications total,
 including application
 described below: 1

45 U.S. Patent Application Serial No. 08/367,395
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SEQUENCE LISTING

5 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 3517 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 DESCRIPTION: Rabbit polyimmunoglobulin receptor

15 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 124....2445

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```

GGCCGGGGTT ACGGGCTGGC CAGCAGGCTG TGCCCCGAG TCCGGTCAGCAGGAGGGGAA      60
GAAGTGGCCT AAAATCTCTC CCGCATCGGC AGCCCAGGCC TAGTGCCTA CCAGCCACCA      120
GCC ATG GCT CTC TTC TTG CTC ACC TGC CTG CTG GCT GTC TTT TCA GCG      168
  1   5   10   15
Met Ala Leu Phe Leu Thr Cys Leu Leu Ala Val Phe Ser Ala
GCC ACG GCA CAA AGC TCC TTA TTG GGT CCC AGC TCC ATA TTT GGT CCC      216
Ala Thr Ala Gln Ser Ser Leu Leu Gly Pro Ser Ser Ile Phe Gly Pro
  20   25   30
GGG GAG GTG AAT GTT TTG GAA GGC GAC TCG GTG TCC ATC ACA TGC TAC      264
Gly Glu Val Asn Val Leu Glu Gly Asp Ser Val Ser Ile Thr Cys Tyr
  35   40   45
TAC CCA ACA ACC TCC GTC ACC CGG CAC AGC CGG AAG TTC TGG TGC CGG      312
Tyr Pro Thr Ser Val Thr Arg His Ser Arg Lys Phe Trp Cys Arg
  50   55   60
GAA GAG GAG AGC GGC CGC TGC GTG ACG CTT GCC TCG ACC GGC TAC ACG      360
Glu Glu Glu Ser Gly Arg Cys Val Thr Leu Ala Ser Thr Gly Tyr Thr
  65   70   75
TCC CAG GAA TAC TCC GGG AGA GGC AAG CTC ACC GAC TTC CCT GAT AAA      408
Ser Gln Glu Tyr Ser Gly Arg Gly Lys Leu Thr Asp Phe Pro Asp Lys
  80   85   90   95
GGG GAG TTT GTG GTG ACT GTT GAC CAA CTC ACC CAG AAC GAC TCA GGG      456
Gly Glu Phe Val Val Thr Val Asp Gln Leu Thr Gln Asn Asp Ser Gly
  100  105  110
AGC TAC AAG TGT GGC GTG GGA GTC AAC GGC CGT GGC CTG GAC TTC GGT      504
Ser Tyr Lys Cys Gly Val Gly Val Asn Gly Arg Gly Leu Asp Phe Gly
  115  120  125
GTC AAC GTG CTG GTC AGC CAG AAG CCA GAG CCT GAT GAC GTT GTT TAC      552
Val Asn Val Leu Val Ser Gln Lys Pro Glu Pro Asp Asp Val Val Tyr
  130  135  140
AAA CAA TAT GAG AGT TAT ACA GTA ACC ATC ACC TGC CCT TTC ACA TAT      600
Lys Gln Tyr Glu Ser Tyr Thr Val Thr Ile Thr Cys Pro Phe Thr Tyr
  145  150  155

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65

100

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | GCG | ACT | AGG | CAA | CTA | AAG | AAG | TCC | TTT | TAC | AAG | GTG | GAA | GAC | GGG | GAA | 648 |
| | Ala | Thr | Arg | Gln | Leu | Lys | Lys | Ser | Phe | Tyr | Lys | Val | Glu | Asp | Gly | Glu | |
| | 160 | | | | | 165 | | | | | 170 | | | | | 175 | |
| 5 | CTT | GTA | CTC | ATC | ATT | GAT | TCC | AGC | AGT | AAG | GAG | GCA | AAG | GAC | CCC | AGG | 696 |
| | Leu | Val | Leu | Ile | Ile | Asp | Ser | Ser | Ser | Lys | Glu | Ala | Lys | Asp | Pro | Arg | |
| | | | | | 180 | | | | | 185 | | | | | 190 | | |
| 10 | TAT | AAG | GGC | AGA | ATA | ACG | TTG | CAG | ATC | CAA | AGT | ACC | ACA | GCA | AAA | GAA | 744 |
| | Tyr | Lys | Gly | Arg | Ile | Thr | Leu | Gln | Ile | Gln | Ser | Thr | Thr | | Lys | Glu | |
| | | | | 195 | | | | | 200 | | | | | 205 | | | |
| 15 | TTC | ACA | GTC | ACC | ATC | AAG | CAT | TTG | CAG | CTC | AAT | GAT | GCT | GGG | CAG | TAT | 792 |
| | Phe | Thr | Val | Thr | Ile | Lys | His | Leu | Gln | Leu | Asn | Asp | Ala | Gly | Gln | Tyr | |
| | | | 210 | | | | | 215 | | | | | 220 | | | | |
| 20 | GTC | TGC | CAG | AGT | GGA | AGC | GAC | CCC | ACT | GCT | GAA | GAA | CAG | AAC | GTT | GAC | 840 |
| | Val | Cys | Gln | Ser | Gly | Ser | | Pro | Thr | Ala | Glu | Glu | Gln | Asn | Val | Asp | |
| | | 225 | | | | 230 | | | | | 235 | | | | | | |
| 25 | CTC | CGA | CTG | CTA | ACT | CCT | GGT | CTG | CTC | TAT | GGA | AAC | CTG | GGG | GGC | TCG | 888 |
| | Leu | Arg | Leu | Leu | Thr | Pro | Gly | Leu | Leu | Tyr | Gly | Asn | Leu | Gly | Gly | Ser | |
| | 240 | | | | 245 | | | | | 250 | | | | | | 255 | |
| 30 | GTG | ACC | TTT | GAA | TGT | GCC | CTG | GAC | TCT | GAA | GAC | GCA | AAC | GCG | GTA | GCA | 936 |
| | Val | Thr | Phe | Glu | Cys | Ala | Leu | Asp | Ser | Glu | Asp | Ala | Asn | Ala | Val | Ala | |
| | | | | 260 | | | | | | 265 | | | | | 270 | | |
| 35 | TCC | TTG | CGC | CAG | GTT | AGG | GGT | GGC | AAT | GTG | GTC | ATT | GAC | AGC | CAG | GGG | 984 |
| | Ser | Leu | Arg | Gln | Val | Arg | Gly | Gly | Asn | Val | Val | Ile | Asp | Ser | Gln | Gly | |
| | | | | 275 | | | | | 280 | | | | | 285 | | | |
| 40 | ACA | ATA | GAT | CCA | GCC | TTC | GAG | GGC | AGG | ATC | CTG | TTC | ACC | AAG | GCT | GAG | 1032 |
| | Thr | Ile | Asp | Pro | Ala | Phe | Glu | Gly | Arg | Ile | Leu | Phe | Thr | Lys | Ala | Glu | |
| | | | 290 | | | | | 295 | | | | | 300 | | | | |
| 45 | AAC | GGC | CAC | TTC | AGT | GTA | GTG | ATC | GCA | GGC | CTG | AGG | AAG | GAA | GAC | ACA | 1080 |
| | Asn | Gly | His | Phe | Ser | Val | Val | Ile | Ala | Gly | Leu | Arg | Lys | Glu | Asp | Thr | |
| | | 305 | | | | 310 | | | | | | 315 | | | | | |
| 50 | GGG | AAC | TAT | CTG | TGC | GGA | GTC | CAG | TCC | AAT | GGT | CAG | TCT | GGG | GAT | GGG | 1128 |
| | Gly | Asn | Tyr | Leu | Cys | Gly | Val | Gln | Ser | Asn | Gly | Gln | Ser | Gly | Asp | Gly | |
| | 320 | | | | 325 | | | | | 330 | | | | | 335 | | |
| 55 | CCC | ACC | CAG | CTT | CGG | CAA | CTC | TTC | GTC | AAT | GAA | GAG | ATC | GAC | GTG | TCC | 1176 |
| | Pro | Thr | Gln | Leu | Arg | Gln | Leu | Phe | Val | Asn | Glu | Glu | Ile | Asp | Val | Ser | |
| | | | | 340 | | | | | 345 | | | | | | 350 | | |
| 60 | CGC | AGC | CCC | CCT | GTG | TTG | AAG | GGC | TTT | CCA | GGA | GGC | TCC | GTG | ACC | ATA | 1224 |
| | Arg | Ser | Pro | Pro | Val | Leu | Lys | Gly | Phe | Pro | Gly | Gly | Ser | Val | Thr | Ile | |
| | | | | 355 | | | | | 360 | | | | | 365 | | | |
| 65 | CGC | TGC | CCC | TAC | AAC | CCG | AAG | AGA | AGC | GAC | AGC | CAC | CTG | CAG | CTG | TAT | 1272 |
| | Arg | Cys | Pro | Tyr | Asn | Pro | Lys | Arg | Ser | Asp | Ser | His | Leu | Gln | Leu | Tyr | |
| | | | 370 | | | | 375 | | | | | | 380 | | | | |
| 70 | CTC | TGG | GAA | GGG | AGT | CAA | ACC | CGC | CAT | CTG | CTG | GTG | GAC | AGC | GGC | GAG | 1320 |
| | Leu | Trp | Glu | Gly | Ser | Gln | Thr | Arg | His | Leu | Leu | Val | Asp | Ser | Gly | Glu | |
| | | 385 | | | | 390 | | | | | | 395 | | | | | |
| 75 | GGG | CTG | GTT | CAG | AAA | GAC | TAC | ACA | GGC | AGG | CTG | GCC | CTG | TTC | GAA | GAG | 1368 |
| | Gly | Leu | Val | Gln | Lys | Asp | Tyr | Thr | Gly | Arg | Leu | Ala | Leu | Phe | Glu | Glu | |
| | 400 | | | | 405 | | | | | 410 | | | | | | 415 | |

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| | | |
|----|---|------|
| | CCT GGC AAT GGC ACC TTC TCA GTC GTC CTC AAC CAG CTC ACT GCC GAG | 1416 |
| | Pro Gly Asn Gly Thr Phe Ser Val Val Leu Asn Gln Leu Thr Ala Glu | |
| | 420 425 430 | |
| 5 | GAT GAA GGC TTC TAC TGG TGT GTC AGC GAT GAC GAT GAG TCC CTG ACG | 1464 |
| | Asp Glu Gly Phe Tyr Trp Cys Val Ser Asp Asp Asp Glu Ser Leu Thr | |
| | 435 440 445 | |
| 10 | ACT TCG GTG AAG CTC CAG ATC GTT GAC GGA GAA CCA AGC CCC ACG ATC | 1512 |
| | Thr Ser Val Lys Leu Gln Ile Val Asp Gly Glu Pro Ser Pro Thr Ile | |
| | 450 455 460 | |
| 15 | GAC AAG TTC ACT GCT GTG CAG GGA GAG CCT GTT GAG ATC ACC TGC CAC | 1560 |
| | Asp Lys Phe Thr Ala Val Gln Gly Glu Pro Val Glu Ile Thr Cys His | |
| | 465 470 475 | |
| 20 | TTC CCA TGC AAA TAC TTC TCC TCC GAG AAG TAC TGG TGC AAG TGG AAT | 1608 |
| | Phe Pro Cys Lys Tyr Phe Ser Ser Glu Lys Tyr Trp Cys Lys Trp Asn | |
| | 480 485 490 495 | |
| | GAC CAT GGC TGC GAG GAC CTG CCC ACT AAG CTC AGC TCC AGC GGC GAC | 1656 |
| | Asp His Gly Cys Glu Asp Leu Pro Thr Lys Leu Ser Ser Ser Gly Asp | |
| | 500 505 510 | |
| 25 | CTT GTG AAA TGC AAC AAC AAC CTG GTC CTC ACC CTG ACC TTG GAC TCG | 1704 |
| | Leu Val Lys Cys Asn Asn Asn Leu Val Leu Thr Leu Thr Leu Asp Ser | |
| | 515 520 525 | |
| 30 | GTC AGC GAA GAT GAC GAG GGC TGG TAC TGG TGT GGC GCG AAA GAC GGG | 1752 |
| | Val Ser Glu Asp Asp Glu Gly Trp Tyr Trp Cys Gly Ala Lys Asp Gly | |
| | 530 535 540 | |
| 35 | CAC GAG TTT GAA GAG GTT GCG GCC GTC AGG GTG GAG CTG ACA GAG CCA | 1800 |
| | His Glu Phe Glu Glu Val Ala Ala Val Arg Val Glu Leu Thr Glu Pro | |
| | 545 550 555 | |
| 40 | GCC AAG GTA GCT GTC GAG CCA GCC AAG GTA CCT GTC GAC CCA GCC AAG | 1848 |
| | Ala Lys Val Ala Val Glu Pro Ala Lys Val Pro Val Asp Pro Ala Lys | |
| | 560 565 570 575 | |
| | GCA GCC CCC GCG CCT GCT GAG GAG AAG GCC AAG GCG CGG TGC CCA GTG | 1896 |
| | Ala Ala Pro Ala Pro Ala Glu Glu Lys Ala Lys Ala Arg Cys Pro Val | |
| | 580 585 590 | |
| 45 | CCC AGG AGA AGG CAG TGG TAC CCA TTG TCA AGG AAG CTG AGA ACA AGT | 1944 |
| | Pro Arg Arg Arg Gln Trp Tyr Pro Leu Ser Arg Lys Leu Arg Thr Ser | |
| | 595 600 605 | |
| 50 | TGT CCA GAA CCT CGG CTC CTT GCG GAG GAG GTA GCA GTG CAG AGT GCG | 1992 |
| | Cys Pro Glu Pro Arg Leu Leu Ala Glu Glu Val Ala Val Gln Ser Ala | |
| | 610 615 620 | |
| 55 | GAA GAC CCA GCC AGT GGG AGC AGA GCG TCT GTG GAT GCC AGC AGT GCT | 2040 |
| | Glu Asp Pro Ala Ser Gly Ser Arg Ala Ser Val Asp Ala Ser Ser Ala | |
| | 625 630 635 | |
| 60 | TCG GGA CAA AGC GGG AGT GCC AAA GTA CTG ATC TCC ACC CTG GTG CCC | 2088 |
| | Ser Gly Gln Ser Gly Ser Ala Lys Val Leu Ile Ser Thr Leu Val Pro | |
| | 640 645 650 655 | |
| | TTG GGG CTG GTG CTG GCA GCG GGG GCC ATG GCC GTG GCC ATA GCC AGA | 2136 |
| | Leu Gly Leu Val Leu Ala Ala Gly Ala Met Ala Val Ala Ile Ala Arg | |
| | 660 665 670 | |

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| | | |
|----|--|------|
| | GCC CGG CAC AGG AGG AAC GTG GAC CGA GTT TCC ATC GGA AGC TAC AGG | 2184 |
| | Ala Arg His Arg Arg Asn Val Asp Arg Val Ser Ile Gly Ser Tyr Arg | |
| | 675 680 685 | |
| 5 | ACA GAC ATT AGC ATG TCA GAC TTG GAG AAC TCC AGG GAG TTC GGA GCC | 2232 |
| | Thr Asp Ile Ser Met Ser Asp Leu Glu Asn Ser Arg Glu Phe Gly Ala | |
| | 690 695 700 | |
| 10 | ATT GAC AAC CCA AGC GCC TGC CCC GAT GCC CGG GAG ACG GCC CTC GGA | 2280 |
| | Ile Asp Asn Pro Ser Ala Cys Pro Asp Ala Arg Glu Thr Ala Leu Gly | |
| | 705 710 715 | |
| 15 | GGA AAG GAT GAG TTA GCG ACG GCC ACC GAG AGC ACC GTG GAG ATT GAG | 2328 |
| | Gly Lys Asp Glu Leu Ala Thr Ala Thr Glu Ser Thr Val Glu Ile Glu | |
| | 720 725 730 735 | |
| 20 | GAG CCC AAG AAG GCA AAA CGG TCA TCC AAG GAA GAA GCC GAC CTG GCC | 2376 |
| | Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu Ala Asp Leu Ala | |
| | 740 745 750 | |
| | TAC TCA GCT TTC CTG CTC CAA TCC AAC ACC ATA GCT GCT GAG CAC CAA | 2424 |
| | Tyr Ser Ala Phe Leu Leu Gln Ser Asn Thr Ile Ala Ala Glu His Gln | |
| | 755 760 765 | |
| 25 | GAT GGC CCC AAG GAG GCC TAG GCACAGCCGG CCACCGCCGC CGCCGCCACC GCCGC | 2480 |
| | Asp Gly Pro Lys Glu Ala | |
| | 770 | |
| 30 | CGCCGCCGCC ACCTGTGAAA ATCACCTTCC AGAATCACGT TGATCCTCGG GGTCCCCAGA | 2540 |
| | GCCGGGGGGCT CAACCGCCCT GCACCCCCCA TGTCCCCACC ACCTAAACTT CCCTACCTGT | 2600 |
| | GCCCAGAGGT GTGCTGGTCC CCTCTCCAC GGCATCCAGG CCTGGCTCAA TGTTCCCGTT | 2660 |
| 35 | GGGGTGGGGG TGTGAGGGGT TCCTACTTGC AGCCCGGTTT TCCCGAGAGA AGCTAAGGAT | 2720 |
| | CCAGGTCTTG AGGGAGGGGC CTCTCGAAGG CAGACAGACC AGAGAGGGGG GAGGAGCCCT | 2780 |
| 40 | TGGATGGGAG GCCAGAGGCG CTTTCCGGCC ACCCCTCCC TCCCTGCCCC CACCCTCCTT | 2840 |
| | CCTTCATTCA AAAGTCCCAG TGGCTGCTGC CTAGGGTCCA GCGCTGGCC GCACGCTCC | 2900 |
| | TCAAGCCGT TGTGCAAACA TCACTGGAGG AAGCCAGGGC TCCTCCCGGG CTGTGTATCC | 2960 |
| 45 | TCACTCAGGC ATCCTGTCCT CCCAGTATC AGGAGATGTC AAGCGTCTGA AGGCTGTGTG | 3020 |
| | CCCTGGGCGT GTCTGCAAGT CACCCCAGAC ACATGTTCTC GCCATTTTAC AGATGAGAAC | 3080 |
| 50 | ACTGAGGTTG TACTCAAGGG CACCCTGCGA GATGGAGCAA CAGCAAATA GATGGGCTTC | 3140 |
| | TGCTGTCCTC TTGGCCAGAG GTCTCTCCAC AGGAGCCCCT GCCCTGTAG GAAGCAGAGT | 3200 |
| | TTTAGAACAT GGAAGAAGAA GAGGGGGATG GCCCTGGACG CTGACCTCTC CCAAGCCCCC | 3260 |
| 55 | ACGGGGGAAA AGGCCCCCTC CTTTTCTGTC ACTCTCGGGG ACCTGCGGAG TTGAGCATTC | 3320 |
| | GTGCCCCGTG TGTCTGAAGA GTTCCCAGTG GAAAGAAGAA AAGAGGGTGT TTGTCACTGC | 3380 |
| 60 | CGGGGAGGGC CTGATCCCCA GACAGCTGAA GTTTAAGGTC CTTGTCCCTG TGAGCTTTAA | 3440 |
| | CCAGCACCTC CGGGCTGACC CTTGCTAACA CATCAGAAAT GTGATTTAAT CATTAAACAT | 3500 |
| | TGTGATTGCC ACTGGGA | 3517 |
| 65 | | |

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 773 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear
 10 DESCRIPTION: Rabbit polyimmunoglobulin receptor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Leu Phe Leu Leu Thr Cys Leu Leu Ala Val Phe Ser Ala Ala
 1 5 10 15
 15 Thr Ala Gln Ser Ser Leu Leu Gly Pro Ser Ser Ile Phe Gly Pro Gly
 20 25 30
 20 Glu Val Asn Val Leu Glu Gly Asp Ser Val Ser Ile Thr Cys Tyr Tyr
 35 40 45
 Pro Thr Thr Ser Val Thr Arg His Ser Arg Lys Phe Trp Cys Arg Glu
 50 55 60
 25 Glu Glu Ser Gly Arg Cys Val Thr Leu Ala Ser Thr Gly Tyr Thr Ser
 65 70 75 80
 Gln Glu Tyr Ser Gly Arg Gly Lys Leu Thr Asp Phe Pro Asp Lys Gly
 85 90 95
 30 Glu Phe Val Val Thr Val Asp Gln Leu Thr Gln Asn Asp Ser Gly Ser
 100 105 110
 35 Tyr Lys Cys Gly Val Gly Val Asn Gly Arg Gly Leu Asp Phe Gly Val
 115 120 125
 Asn Val Leu Val Ser Gln Lys Pro Glu Pro Asp Asp Val Val Tyr Lys
 130 135 140
 40 Gln Tyr Glu Ser Tyr Thr Val Thr Ile Thr Cys Pro Phe Thr Tyr Ala
 145 150 155 160
 Thr Arg Gln Leu Lys Lys Ser Phe Tyr Lys Val Glu Asp Gly Glu Leu
 165 170 175
 45 Val Leu Ile Ile Asp Ser Ser Ser Lys Glu Ala Lys Asp Pro Arg Tyr
 180 185 190
 50 Lys Gly Arg Ile Thr Leu Gln Ile Gln Ser Thr Thr Ala Lys Glu Phe
 195 200 205
 Thr Val Thr Ile Lys His Leu Gln Leu Asn Asp Ala Gly Gln Tyr Val
 210 215 220
 55 Cys Gln Ser Gly Ser Asp Pro Thr Ala Glu Glu Gln Asn Val Asp Leu
 225 230 235 240
 Arg Leu Leu Thr Pro Gly Leu Leu Tyr Gly Asn Leu Gly Gly Ser Val
 245 250 255
 60 Thr Phe Glu Cys Ala Leu Asp Ser Glu Asp Ala Asn Ala Val Ala Ser
 260 265 270
 65 Leu Arg Gln Val Arg Gly Gly Asn Val Val Ile Asp Ser Gln Gly Thr
 275 280 285

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Ile Asp Pro Ala Phe Glu Gly Arg Ile Leu Phe Thr Lys Ala Glu Asn
 290 295 300
 5 Gly His Phe Ser Val Val Ile Ala Gly Leu Arg Lys Glu Asp Thr Gly
 305 310 315 320
 Asn Tyr Leu Cys Gly Val Gln Ser Asn Gly Gln Ser Gly Asp Gly Pro
 325 330 335
 10 Thr Gln Leu Arg Gln Leu Phe Val Asn Glu Glu Ile Asp Val Ser Arg
 340 345 350
 Ser Pro Pro Val Leu Lys Gly Phe Pro Gly Gly Ser Val Thr Ile Arg
 355 360 365
 15 Cys Pro Tyr Asn Pro Lys Arg Ser Asp Ser His Leu Gln Leu Tyr Leu
 370 375 380
 20 Trp Glu Gly Ser Gln Thr Arg His Leu Leu Val Asp Ser Gly Glu Gly
 385 390 395 400
 Leu Val Gln Lys Asp Tyr Thr Gly Arg Leu Ala Leu Phe Glu Glu Pro
 405 410 415
 25 Gly Asn Gly Thr Phe Ser Val Val Leu Asn Gln Leu Thr Ala Glu Asp
 420 425 430
 Glu Gly Phe Tyr Trp Cys Val Ser Asp Asp Asp Glu Ser Leu Thr Thr
 435 440 445
 30 Ser Val Lys Leu Gln Ile Val Asp Gly Glu Pro Ser Pro Thr Ile Asp
 450 455 460
 35 Lys Phe Thr Ala Val Gln Gly Glu Pro Val Glu Ile Thr Cys His Phe
 465 470 475 480
 Pro Cys Lys Tyr Phe Ser Ser Glu Lys Tyr Trp Cys Lys Trp Asn Asp
 485 490 495
 40 His Gly Cys Glu Asp Leu Pro Thr Lys Leu Ser Ser Ser Gly Asp Leu
 500 505 510
 Val Lys Cys Asn Asn Asn Leu Val Leu Thr Leu Thr Leu Asp Ser Val
 515 520 525
 45 Ser Glu Asp Asp Glu Gly Trp Tyr Trp Cys Gly Ala Lys Asp Gly His
 530 535 540
 50 Glu Phe Glu Glu Val Ala Ala Val Arg Val Glu Leu Thr Glu Pro Ala
 545 550 555 560
 Lys Val Ala Val Glu Pro Ala Lys Val Pro Val Asp Pro Ala Lys Ala
 565 570 575
 55 Ala Pro Ala Pro Ala Glu Glu Lys Ala Lys Ala Arg Cys Pro Val Pro
 580 585 590
 Arg Arg Arg Gln Trp Tyr Pro Leu Ser Arg Lys Leu Arg Thr Ser Cys
 595 600 605
 60 Pro Glu Pro Arg Leu Leu Ala Glu Glu Val Ala Val Gln Ser Ala Glu
 610 615 620
 65 Asp Pro Ala Ser Gly Ser Arg Ala Ser Val Asp Ala Ser Ser Ala Ser
 625 630 635 640

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[illegible]

30 (2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 2919 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
DESCRIPTION: Human polyimmunoglobulin Receptor

(ix) **FEATURE:**

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 235...2472

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

| | | | | | | | |
|----|---|--|------------|------------|------------|----------------------|-----|
| 50 | AGAGTTTCAG | TTTTGGCAGC | AGCGTCCAGT | GCCCTGCCAG | TAGCTCCTAG | AGAGGCAGGG | 60 |
| | GTTACCAACT | GGCCAGCAGG | CTGTGTCCCT | GAAGTCAGAT | CAACGGGAGA | GAAGGAAGTG | 120 |
| 50 | GCTAAACAT | TGCACAGGAG | AAGTCGGCCT | GAGTGGTGCG | GCGCTCGGGA | CCCACCAGCA | 180 |
| | ATGCTGCTCT | TCGTGCTCAC | CTGCCTGCTG | GCGGTCTTCC | CAGCCATCTC | CACG AAG Lys 1 | 237 |
| 55 | AGT CCC ATA TTT GGT CCC GAG GAG GTG AAT AGT GTG GAA GGT AAC TCA | 285 | | | | | |
| | Ser Pro Ile Phe 5 | Gly Pro Glu Glu Val Asn Ser Val Glu Gly Asn Ser 10 15 | | | | | |
| 60 | GTG TCC ATC ACG TGC TAC TAC CCA CCC ACC TCT GTC AAC CGG CAC ACC | 333 | | | | | |
| | Val Ser Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn Arg His Thr 20 25 30 | | | | | | |
| 65 | CGG AAG TAC TGG TGC CGG CAG GGA GCT AGA GGT GGC TGC ATA ACC CTC | 381 | | | | | |
| | Arg Lys Tyr Trp Cys Arg Gln Gly Ala Arg Gly Gly Cys Ile Thr Leu 35 40 45 | | | | | | |

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| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | ATC | TCC | TCG | GAG | GGC | TAC | GTC | TCC | AGC | AAA | TAT | GCA | GGC | AGG | GCT | AAC | 429 |
| | Ile | Ser | Ser | Glu | Gly | Tyr | Val | Ser | Ser | Lys | Tyr | Ala | Gly | Arg | Ala | Asn | |
| | 50 | | | | | 55 | | | | | 60 | | | | | 65 | |
| 5 | CTC | ACC | AAC | TTC | CCG | GAG | AAC | GGC | ACA | TTT | GTG | GTG | AAC | ATT | GCC | CAG | 477 |
| | Leu | Thr | Asn | Phe | Pro | Glu | Asn | Gly | Thr | Phe | Val | Val | Asn | Ile | Ala | Gln | |
| | | | | | 70 | | | | | 75 | | | | | 80 | | |
| 10 | CTG | AGC | CAG | GAT | GAC | TCC | GGG | CGC | TAC | AAG | TGT | GGC | CTG | GGC | ATC | AAT | 525 |
| | Leu | Ser | Gln | Asp | Asp | Ser | Gly | Arg | Tyr | Lys | Cys | Gly | Leu | Gly | Ile | Asn | |
| | | | | 85 | | | | | 90 | | | | | 95 | | | |
| 15 | AGC | CGA | GGC | CTG | TCC | TTT | GAT | GTC | AGC | CTG | GAG | GTC | AGC | CAG | GGT | CCT | 573 |
| | Ser | Arg | | Gly | Leu | Ser | Phe | Asp | Ser | Leu | Glu | Val | Ser | Gln | Gly | Pro | |
| | | | 100 | | | | | 105 | | | | | 110 | | | | |
| 20 | GGG | CTC | CTA | AAT | GAC | ACT | AAA | GTC | TAC | ACA | GTG | GAC | CTG | GGC | AGA | ACG | 621 |
| | Gly | Leu | Leu | Asn | Asp | Thr | Lys | Val | Tyr | Thr | Val | Asp | Leu | Gly | Arg | Thr | |
| | | 115 | | | | | 120 | | | | | 125 | | | | | |
| 25 | GTG | ACC | ATC | AAC | TGC | CCT | TTC | AAG | ACT | GAG | AAT | GCT | CAA | AAG | AGG | AAG | 669 |
| | Val | Thr | Ile | Asn | Cys | Pro | Phe | Lys | Thr | Glu | Asn | Ala | Gln | Lys | Arg | Lys | |
| | 130 | | | | | 135 | | | | | 140 | | | | | 145 | |
| 30 | TCC | TTG | TAC | AAG | CAG | ATA | GGC | CTG | TAC | CCT | GTG | CTG | GTC | ATC | GAC | TCC | 717 |
| | Ser | Leu | Tyr | Lys | Gln | Ile | Gly | Leu | Tyr | Pro | Val | Leu | Val | Ile | Asp | Ser | |
| | | | | | 150 | | | | | 155 | | | | | 160 | | |
| 35 | AGT | GGT | TAT | GTG | AAT | CCC | AAC | TAT | ACA | GGA | AGA | ATA | CGC | CTT | GAT | ATT | 765 |
| | Ser | Gly | Tyr | Val | Asn | Pro | Asn | Tyr | Thr | Gly | Arg | Ile | Arg | Leu | Asp | Ile | |
| | | | | 165 | | | | 170 | | | | | | 175 | | | |
| 40 | CAG | GGT | ACT | GGC | CAG | TTA | CTG | TTC | AGC | GTT | GTC | ATC | AAC | CAA | CTC | AGG | 813 |
| | Gln | Gly | Thr | Gly | Gln | Leu | Leu | Phe | Ser | Val | Val | Ile | Asn | Gln | Leu | Arg | |
| | | | 180 | | | | | 185 | | | | | 190 | | | | |
| 45 | CTC | AGC | GAT | GCT | GGG | CAG | TAT | CTC | TGC | CAG | GCT | GGG | GAT | GAT | TCC | AAT | 861 |
| | Leu | Ser | Asp | Ala | Gly | Gln | Tyr | Leu | Cys | Gln | Ala | Gly | Asp | Asp | Ser | Asn | |
| | | 195 | | | | | 200 | | | | | 205 | | | | | |
| 50 | AGT | AAT | AAG | AAG | AAT | GCT | GAC | CTC | CAA | GTG | CTA | AAG | CCC | GAG | CCC | GAG | 909 |
| | Ser | Asn | Lys | Lys | Asn | Ala | Asp | Leu | Gln | Val | Leu | Lys | Pro | Glu | Pro | Glu | |
| | 210 | | | | 215 | | | | | 220 | | | | | | 225 | |
| 55 | CTG | GTT | TAT | GAA | GAC | CTG | AGG | GGC | TCA | GTG | ACC | TTC | CAC | TGT | GCC | CTG | 957 |
| | Leu | Val | Tyr | Glu | Asp | Leu | Arg | Gly | Ser | Val | Thr | Phe | His | Cys | Ala | Leu | |
| | | | | | 230 | | | | | 235 | | | | | 240 | | |
| 60 | GGC | CCT | GAG | GTG | GCA | AAC | GTG | GCC | AAA | TTT | CTG | TGC | CGA | CAG | AGC | AGT | 1005 |
| | Gly | Pro | Glu | Val | Ala | Asn | Val | Ala | Lys | Phe | Leu | Cys | Arg | Gln | Ser | Ser | |
| | | | | 245 | | | | | 250 | | | | | 255 | | | |
| 65 | GGG | GAA | AAC | TGT | GAC | GTG | GTC | GTC | AAC | ACC | CTG | GGG | AAG | AGG | GCC | CCA | 1053 |
| | Gly | Glu | Asn | Cys | Asp | Val | Val | Val | Asn | Thr | Leu | Gly | Lys | Arg | Ala | Pro | |
| | | | 260 | | | | | 265 | | | | | 270 | | | | |
| 70 | GCC | TTT | GAG | GGC | AGG | ATC | CTG | CTC | AAC | CCC | CAG | GAC | AAG | GAT | GGC | TCA | 1101 |
| | Ala | Phe | Glu | Gly | Arg | Ile | Leu | Leu | Asn | Pro | Gln | Asp | Lys | Asp | Gly | Ser | |
| | | 275 | | | | | 280 | | | | | 285 | | | | | |
| 75 | TTC | AGT | GTG | GTG | ATC | ACA | GGC | CTG | AGG | AAG | GAG | GAT | GCA | GGG | CGC | TAC | 1149 |
| | Phe | Ser | Val | Val | Ile | Thr | Gly | Leu | Arg | Lys | Glu | Asp | Ala | Gly | Arg | Tyr | |
| | 290 | | | | | 295 | | | | | 300 | | | | | 305 | |

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| | | |
|----|---|------|
| | CTG TGT GGA GCC CAT TCG GAT GGT CAG CTG CAG GAA GGC TCG CCT ATC | 1197 |
| | Leu Cys Gly Ala His Ser Asp Gly Gln Leu Gln Glu Gly Ser Pro Ile | |
| | 310 315 320 | |
| 5 | CAG GCC TGG CAA CTC TTC GTC AAT GAG GAG TCC ACG ATT CCC CGC AGC | 1245 |
| | Gln Ala Trp Gln Leu Phe Val Asn Glu Ser Thr Ile Pro Arg Ser | |
| | 325 330 335 | |
| 10 | CCC ACT GTG GTG AAG GGG GTG GCA GGA AGC TCT GTG GCC GTG CTC TGC | 1293 |
| | Pro Thr Val Val Lys Gly Val Ala Gly Ser Ser Val Ala Val Leu Cys | |
| | 340 345 350 | |
| 15 | CCC TAC AAC CGT AAG GAA AGC AAA AGC ATC AAG TAC TGG TGT CTC TGG | 1341 |
| | Pro Tyr Asn Arg Lys Glu Ser Lys Ser Ile Lys Tyr Trp Cys Leu Trp | |
| | 355 360 365 | |
| 20 | GAA GGG GCC CAG AAT GGC CGC TGC CCC CTG CTG GTG GAC AGC GAG GGG | 1389 |
| | Glu Gly Ala Gln Asn Gly Arg Cys Pro Leu Leu Val Asp Ser Glu Gly | |
| | 370 375 380 385 | |
| | TGG GTT AAG GCC CAG TAC GAG GGC CGC CTC TCC CTG CTG GAG GAG CCA | 1437 |
| | Trp Val Lys Ala Gln Tyr Glu Gly Arg Leu Ser Leu Leu Glu Glu Pro | |
| | 390 395 400 | |
| 25 | GGC AAC GGC ACC TTC ACT GTC ATC CTC AAC CAG CTC ACC AGC CGG GAC | 1485 |
| | Gly Asn Gly Thr Phe Thr Val Ile Leu Asn Gln Leu Thr Ser Arg Asp | |
| | 405 410 415 | |
| 30 | GCC GGC TTC TAC TGG TGT CTG ACC AAC GGC GAT ACT CTC TGG AGG ACC | 1533 |
| | Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Thr Leu Trp Arg Thr | |
| | 420 425 430 | |
| 35 | ACC GTG GAG ATC AAG ATT ATC GAA GGA GAA CCA AAC CTC AAG GTA CCA | 1581 |
| | Thr Val Glu Ile Lys Ile Ile Glu Gly Glu Pro Asn Leu Lys Val Pro | |
| | 435 440 445 | |
| 40 | GGG AAT GTC ACG GCT GTG CTG GGA GAG ACT CTC AAG GTC CCC TGT CAC | 1629 |
| | Gly Asn Val Thr Ala Val Leu Gly Glu Thr Leu Lys Val Pro Cys His | |
| | 450 455 460 465 | |
| | TTT CCA TGC AAA TTC TCC TCG TAC GAG AAA TAC TGG TGC AAG TGG AAT | 1677 |
| | Phe Pro Cys Lys Phe Ser Ser Tyr Glu Lys Tyr Trp Cys Lys Trp Asn | |
| | 470 475 480 | |
| 45 | AAC ACG GGC TGC CAG GCC CTG CCC AGC CAA GAC GAA GGC CCC AGC AAG | 1725 |
| | Asn Thr Gly Cys Gln Ala Leu Pro Ser Gln Asp Glu Gly Pro Ser Lys | |
| | 485 490 495 | |
| 50 | GCC TTC GTG AAC TGT GAC GAG AAC AGC CGG CTT GTC TCC CTG ACC CTG | 1773 |
| | Ala Phe Val Asn Cys Asp Glu Asn Ser Arg Leu Val Ser Leu Thr Leu | |
| | 500 505 510 | |
| 55 | AAC CTG GTG ACC AGG GCT GAT GAG GGC TGG TAC TGG TGT GGA GTG AAG | 1821 |
| | Asn Leu Val Thr Arg Ala Asp Glu Gly Trp Tyr Trp Cys Gly Val Lys | |
| | 515 520 525 | |
| 60 | CAG GGC CAC TTC TAT GGA GAG ACT GCA GCC GTC TAT GTG GCA GTT GAA | 1869 |
| | Gln Gly His Phe Tyr Gly Glu Thr Ala Ala Val Tyr Val Ala Val Glu | |
| | 530 535 540 545 | |
| | GAG AGG AAG GCA GCG GGG TCC CGC GAT GTC AGC CTA GCG AAG GCA GAC | 1917 |
| | Glu Arg Lys Ala Ala Gly Ser Arg Asp Val Ser Leu Ala Lys Ala Asp | |
| | 550 555 560 | |

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|----|--|------|
| | GCT GCT CCT GAT GAG AAG GTG CTA GAC TCT GGT TTT CGG GAG ATT GAG | 1965 |
| | Ala Ala Pro Asp Glu Lys Val Leu Asp Ser Gly Phe Arg Glu Ile Glu | |
| | 565 570 575 | |
| 5 | AAC AAA GCC ATT CAG GAT CCC AGG CTT TTT GCA GAG GAA AAG GCG GTG | 2013 |
| | Asn Lys Ala Ile Gln Asp Pro Arg Leu Phe Ala Glu Glu Lys Ala Val | |
| | 580 585 590 | |
| 10 | GCA GAT ACA AGA GAT CAA GCC GAT GGG AGC AGA GCA TCT GTG GAT TCC | 2061 |
| | Ala Asp Thr Arg Asp Gln Ala Asp Gly Ser Arg Ala Ser Val Asp Ser | |
| | 595 600 605 | |
| 15 | GGC AGC TCT GAG GAA CAA GGT GGA AGC TCC AGA GCG CTG GTC TCC ACC | 2109 |
| | Gly Ser Ser Glu Glu Gln Gly Gly Ser Ser Arg Ala Leu Val Ser Thr | |
| | 610 615 620 625 | |
| 20 | CTG GTG CCC CTG GGC CTG GTG CTG GCA GTG GGA GCC GTG GCT GTG GGG | 2157 |
| | Leu Val Pro Leu Glu Leu Val Leu Ala Val Gly Ala Val Ala Val Gly | |
| | 630 635 640 | |
| | GTG GCC AGA GCC CGG CAC AGG AAG AAC GTC GAC CGA GTT TCA ATC AGA | 2205 |
| | Val Ala Arg Ala Arg His Arg Lys Asn Val Asp Arg Val Ser Ile Arg | |
| | 645 650 655 | |
| 25 | AGC TAC AGG ACA GAC ATT AGC ATG TCA GAC TTC GAG AAC TCC AGG GAA | 2253 |
| | Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu Asn Ser Arg Glu | |
| | 660 665 670 | |
| 30 | TTT GGA GCC AAT GAC AAC ATG GGA GCC TCT TCG ATC ACT CAG GAG ACA | 2301 |
| | Phe Gly Ala Asn Asp Asn Met Gly Ala Ser Ser Ile Thr Gln Glu Thr | |
| | 675 680 685 | |
| 35 | TCC CTC GGA GGA AAA GAA GAG TTT GTT GCC ACC ACT GAG AGC ACC ACA | 2349 |
| | Ser Leu Gly Gly Lys Glu Glu Phe Val Ala Thr Thr Glu Ser Thr Thr | |
| | 690 695 700 705 | |
| 40 | GAG ACC AAA GAA CCC AAG AAG GCA AAA AGG TCA TCC AAG GAG GAA GCC | 2397 |
| | Glu Thr Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu Ala | |
| | 710 715 720 | |
| | GAG ATG GCC TAC AAA GAC TTC CTG CTC CAG TCC AGC ACC GTG GCC GCC | 2445 |
| | Glu Met Ala Tyr Lys Asp Phe Leu Leu Gln Ser Ser Thr Val Ala Ala | |
| | 725 730 735 | |
| 45 | GAG GCC CAG GAC GGC CCC CAG GAA GCC TAGACGGTGT CGCCGCCTGC TCCCTGCA | 2500 |
| | Glu Ala Gln Asp Gly Pro Gln Glu Ala | |
| | 740 745 | |
| 50 | CCCATGACAA TCACCTTCAG AATCATGTCG ATCCTGGGGG CCCTCAGCTC CTGGGGACCC | 2560 |
| | CACTCCCTGC TCTAACACCT GCCTAGGTTT TTCCTACTGT CCTCAGAGGC GTGCTGGTCC | 2620 |
| | CCTCCTCAGT GACATCAAAG CCTGGCCTAA TTGTTCTTAT TGGGGATGAG GGTGGCATGA | 2680 |
| 55 | GGAGGTCCCA CTTGCAACTT CTTTCTGTTG AGAGAACCTC AGGTACGGAG AAGAATAGAG | 2740 |
| | GTCCTCATGG GTCCCTTGAA GGAAGAGGGA CCAGGGTGGG AGAGCTGATT GCAGAAAGGA | 2800 |
| 60 | GAGACGTGCA GCGCCCCTCT GCACCCCTTAT CATGGGATGT CAACAGAATT TTTTCCCTCC | 2860 |
| | ACTCCATCCC TCCCTCCCGT CCTTCCCCTC TTCTTCTTTC CTTACCATCA AAAGATGTA | 2919 |

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 746 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear

DESCRIPTION: Human Polyimmunoglobulin Receptor
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

Lys Ser Pro Ile Phe Gly Pro Glu Glu Val Asn Ser Val Glu Gly Asn
 1 5 10 15

15 Ser Val Ser Ile Thr Cys Tyr Tyr Pro Pro Thr Ser Val Asn Arg His
 20 25 30

Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Arg Gly Gly Cys Ile Thr
 35 40 45

20 Leu Ile Ser Ser Glu Gly Tyr Val Ser Ser Lys Tyr Ala Gly Arg Ala
 50 55 60

25 Asn Leu Thr Asn Phe Pro Glu Asn Gly Thr Phe Val Val Asn Ile Ala
 65 70 75 80

Gln Leu Ser Gln Asp Asp Ser Gly Arg Tyr Lys Cys Gly Leu Gly Ile
 85 90 95

30 Asn Ser Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val Ser Gln Gly
 100 105 110

Pro Gly Leu Leu Asn Asp Thr Lys Val Tyr Thr Val Asp Leu Gly Arg
 115 120 125

35 Thr Val Thr Ile Asn Cys Pro Phe Lys Thr Glu Asn Ala Gln Lys Arg
 130 135 140

40 Lys Ser Leu Tyr Lys Gln Ile Gly Leu Tyr Pro Val Leu Val Ile Asp
 145 150 155 160

Ser Ser Gly Tyr Val Asn Pro Asn Tyr Thr Gly Arg Ile Arg Leu Asp
 165 170 175

45 Ile Gln Gly Thr Gly Gln Leu Leu Phe Ser Val Val Ile Asn Gln Leu
 180 185 190

Arg Leu Ser Asp Ala Gly Gln Tyr Leu Cys Gln Ala Gly Asp Asp Ser
 195 200 205

50 Asn Ser Asn Lys Lys Asn Ala Asp Leu Gln Val Leu Lys Pro Glu Pro
 210 215 220

Glu Leu Val Tyr Glu Asp Leu Arg Gly Ser Val Thr Phe His Cys Ala
 225 230 235 240

Leu Gly Pro Glu Val Ala Asn Val Ala Lys Phe Leu Cys Arg Gln Ser
 245 250 255

60 Ser Gly Glu Asn Cys Asp Val Val Val Asn Thr Leu Gly Lys Arg Ala
 260 265 270

Pro Ala Phe Glu Gly Arg Ile Leu Leu Asn Pro Gln Asp Lys Asp Gly
 275 280 285

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110

Ser Phe Ser Val Val Ile Thr Gly Leu Arg Lys Glu Asp Ala Gly Arg
 290 295 300
 5 Tyr Leu Cys Gly Ala His Ser Asp Gly Gln Leu Gln Glu Gly Ser Pro
 305 310 315 320
 Ile Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile Pro Arg
 325 330 335
 10 Ser Pro Thr Val Val Lys Gly Val Ala Gly Ser Ser Val Ala Val Leu
 340 345 350
 Cys Pro Tyr Asn Arg Lys Glu Ser Lys Ser Ile Lys Tyr Trp Cys Leu
 355 360 365
 15 Trp Glu Gly Ala Gln Asn Gly Arg Cys Pro Leu Leu Val Asp Ser Glu
 370 375 380
 20 Gly Trp Val Lys Ala Gln Tyr Glu Gly Arg Leu Ser Leu Leu Glu Glu
 385 390 395 400
 Pro Gly Asn Gly Thr Phe Thr Val Ile Leu Asn Gln Leu Thr Ser Arg
 405 410 415
 25 Asp Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Thr Leu Trp Arg
 420 425 430
 Thr Thr Val Glu Ile Lys Ile Ile Glu Gly Glu Pro Asn Leu Lys Val
 435 440 445
 30 Pro Gly Asn Val Thr Ala Val Leu Gly Glu Thr Leu Lys Val Pro Cys
 450 455 460
 35 His Phe Pro Cys Lys Phe Ser Ser Tyr Glu Lys Tyr Trp Cys Lys Trp
 465 470 475 480
 Asn Asn Thr Gly Cys Gln Ala Leu Pro Ser Gln Asp Glu Gly Pro Ser
 485 490 495
 40 Lys Ala Phe Val Asn Cys Asp Glu Asn Ser Arg Leu Val Ser Leu Thr
 500 505 510
 Leu Asn Leu Val Thr Arg Ala Asp Glu Gly Trp Tyr Trp Cys Gly Val
 515 520 525
 45 Lys Gln Gly His Phe Tyr Gly Glu Thr Ala Ala Val Tyr Val Ala Val
 530 535 540
 50 Glu Glu Arg Lys Ala Ala Gly Ser Arg Asp Val Ser Leu Ala Lys Ala
 545 550 555 560
 Asp Ala Ala Pro Asp Glu Lys Val Leu Asp Ser Gly Phe Arg Glu Ile
 565 570 575
 55 Glu Asn Lys Ala Ile Gln Asp Pro Arg Leu Phe Ala Glu Glu Lys Ala
 580 585 590
 Val Ala Asp Thr Arg Asp Gln Ala Asp Gly Ser Arg Ala Ser Val Asp
 595 600 605
 60 Ser Gly Ser Ser Glu Glu Gln Gly Gly Ser Ser Arg Ala Leu Val Ser
 610 615 620
 65 Thr Leu Val Pro Leu Gly Leu Val Leu Ala Val Gly Ala Val Ala Val
 625 630 635 640

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Gly Val Ala Arg Ala Arg His Arg Lys Asn Val Asp Arg Val Ser Ile
645 650 655

5 Arg Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu Asn Ser Arg
660 665 670

Glu Phe Gly Ala Asn Asp Asn Met Gly Ala Ser Ser Ile Thr Gln Glu
675 680 685

10 Thr Ser Leu Gly Gly Lys Glu Glu Phe Val Ala Thr Thr Glu Ser Thr
690 695 700

Thr Glu Thr Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu
705 710 715 720

15 Ala Glu Met Ala Tyr Lys Asp Phe Leu Leu Gln Ser Ser Thr Val Ala
725 730 735

20 Ala Glu Ala Gln Asp Gly Pro Gln Glu Ala
740 745

(2) INFORMATION FOR SEQ ID NO: 5:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
30 DESCRIPTION: Bovine Polyimmunoglobulin Receptor

(ix) FEATURE:

35 (A) NAME/KEY: Coding Sequence
(B) LOCATION: 152....2425

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

40 GATCTCCTCG GAGGGTCGTG CAGCGGCCCT GGGTCCCTGC CGGCACCAGT ACTTGCGCGT 60
GTGCTCCCAA AGCTGACGGG ATAGGAGGAA GGAGCTCAAA CAACCACACA GGACGGTGGC 120

45 TGGCGGCAGA GACCCGCGGG AGCCCCCAGC G ATG TCG CGC CTG TTC CTC GCC 172
Met Ser Arg Leu Phe Leu Ala
1 5

50 TGC CTG CTG GCC ATC TTC CCA GTG GTC TCC ATG AAG AGT CCC ATC TTC 220
Cys Leu Leu Ala Ile Phe Pro Val Val Ser Met Lys Ser Pro Ile Phe
10 15 20

55 GGT CCC GAG GAG GTG AGC AGC GTG GAA GGC CGC TCA GTG TCC ATC AAG 268
Gly Pro Glu Glu Val Ser Ser Val Glu Gly Arg Ser Val Ser Ile Lys
25 30 35

60 TGC TAC TAC CCG CCC ACC TCC GTC AAC CGG CAC ACG CGC AAG TAC TGG 316
Cys Tyr Tyr Pro Pro Thr Ser Val Asn Arg His Thr Arg Lys Tyr Trp
40 45 50 55

60 TGC CGG CAG GGA GCC CAG GGC CGC TGC ACG ACC CTC ATC TCC TCG GAG 364
Cys Arg Gln Gly Ala Gln Gly Arg Cys Thr Thr Leu Ile Ser Ser Glu
60 65 70

65 GGC TAC GTC TCC GAC GAC TAC GTG GGC AGA GCC AAC CTC ACC AAC TTC 412
Gly Tyr Val Ser Asp Asp Tyr Val Gly Arg Ala Asn Leu Thr Asn Phe
75 80 85

112

| | | |
|----|---|------|
| | CCG GAG AGC GGC ACG TTT GTG GTG GAC ATC AGC CAT CTC ACC CAT AAA | 460 |
| | Pro Glu Ser Gly Thr Phe Val Val Asp Ile Ser His Leu Thr His Lys | |
| | 90 95 100 | |
| 5 | GAC TCA GGG CGC TAC AAG TGT GGC CTG GGC ATT AGC AGC CGT GGC CTT | 508 |
| | Asp Ser Gly Arg Tyr Lys Cys Gly Leu Gly Ile Ser Ser Arg Gly Leu | |
| | 105 110 115 | |
| 10 | AAC TTC GAT GTG AGC CTG GAG GTC AGC CAA GAT CCT GCA CAG GCA AGT | 556 |
| | Asn Phe Asp Val Ser Leu Glu Val Ser Gln Asp Pro Ala Gln Ala Ser | |
| | 120 125 130 135 | |
| 15 | CAT GCC CAC GTC TAC ACT ATA GAC CTG GGC AGG ACT GTG ACC ATC AAC | 604 |
| | His Ala His Val Tyr Thr Ile Asp Leu Gly Arg Thr Val Thr Ile Asn | |
| | 140 145 150 | |
| 20 | TGC CCT TTC ACG CGT GCG AAT TCT GAG AAG AGA AAA TCC TTG TGC AAG | 652 |
| | Cys Pro Phe Thr Arg Ala Asn Ser Glu Lys Arg Lys Ser Leu Cys Lys | |
| | 155 160 165 | |
| 25 | AAG ACA ATC CAG GAC TGT TTC CAA GTT GTC GAC TCC ACC GGG TAT GTG | 700 |
| | Lys Thr Ile Gln Asp Cys Phe Gln Val Val Asp Ser Thr Gly Tyr Val | |
| | 170 175 180 | |
| 30 | AGC AAC AGC TAT AAA GAC AGA GCA CAT ATC AGT ATC CTA GGT ACC AAC | 748 |
| | Ser Asn Ser Tyr Lys Asp Arg Ala His Ile Ser Ile Leu Gly Thr Asn | |
| | 185 190 195 | |
| 35 | ACA TTA GTG TTC AGC GTT GTC ATC AAC CGA GTC AAG CTC AGT GAT GCT | 796 |
| | Thr Leu Val Phe Ser Val Val Ile Asn Arg Val Lys Leu Ser Asp Ala | |
| | 200 205 210 215 | |
| 40 | GGG ATG TAT GTC TGC CAG GCT GGG GAC GAT GCC AAA GCC GAT AAA ATC | 844 |
| | Gly Met Tyr Val Cys Gln Ala Gly Asp Asp Ala Lys Ala Asp Lys Ile | |
| | 220 225 230 | |
| 45 | AAC ATT GAC CTC CAG GTG CTG GAG CCT GAG CCT GAG CTG GTT TAT GGA | 892 |
| | Asn Ile Asp Leu Gln Val Leu Glu Pro Glu Pro Glu Leu Val Tyr Gly | |
| | 235 240 245 | |
| 50 | GAC TTG AGG AGC TCG GTG ACC TTT GAC TGT TCC CTG GGC CCC GAG GTG | 940 |
| | Asp Leu Arg Ser Ser Val Thr Phe Asp Cys Ser Leu Gly Pro Glu Val | |
| | 250 255 260 | |
| 55 | GCA AAT GTG CCC AAA TTT CTG TGC CAG AAG AAG AAT GGG GGA GCT TGC | 988 |
| | Ala Asn Val Pro Lys Phe Leu Cys Gln Lys Lys Asn Gly Gly Ala Cys | |
| | 265 270 275 | |
| 60 | AAT GTA GTC ATC AAC ACG TTG GGG AAG AAG GCT CAG GAC TTC CAG GGC | 1036 |
| | Asn Val Val Ile Asn Thr Leu Gly Lys Lys Ala Gln Asp Phe Gln Gly | |
| | 280 285 290 295 | |
| 65 | AGG ATC GTG TCC GTG CCC AAG GAC AAT GGT GTC TTC AGT GTG CAC ATT | 1084 |
| | Arg Ile Val Ser Val Pro Lys Asp Asn Gly Val Phe Ser Val His Ile | |
| | 300 305 310 | |
| 70 | ACC AGC CTG AGG AAA GAG GAC GCA GGG CGC TAC GTG TGC GGG GCC CAG | 1132 |
| | Thr Ser Leu Arg Lys Glu Asp Ala Gly Arg Tyr Val Cys Gly Ala Gln | |
| | 315 320 325 | |
| 75 | CCT GAG GGT GAG CCC CAG GAC GGC TGG CCT GTG CAG GCC TGG CAA CTC | 1180 |
| | Pro Glu Gly Glu Pro Gln Asp Gly Trp Pro Val Gln Ala Trp Gln Leu | |
| | 330 335 340 | |

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|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | TTC | GTC | AAT | GAA | GAG | ACG | GCA | ATC | CCC | GCA | AGC | CCC | TCC | GTG | GTG | AAA | 1228 |
| | Phe | Val | Asn | Glu | Glu | Thr | Ala | Ile | Pro | Ala | Ser | Pro | Ser | Val | Val | Lys | |
| | 345 | | | | | | 350 | | | | | 355 | | | | | |
| 5 | GGT | GTG | AGG | GGA | GGC | TCT | GTG | ACT | GTA | TCT | TGC | CCC | TAC | AAC | CCT | AAG | 1276 |
| | Gly | Val | Arg | Gly | Gly | Ser | Val | Thr | Val | Ser | Cys | Pro | Tyr | Asn | Pro | Lys | |
| | 360 | | | | | 365 | | | | | 370 | | | | | 375 | |
| 10 | GAT | GCC | AAC | AGC | GCG | AAG | TAC | TGG | TGT | CAC | TGG | GAA | GAG | GCT | CAA | AAC | 1324 |
| | Asp | Ala | Asn | Ser | Ala | Lys | Tyr | Trp | Cys | His | Trp | Glu | Glu | Ala | Gln | Asn | |
| | | | | | 380 | | | | | 385 | | | | | 390 | | |
| 15 | GGC | CGC | TGC | CCG | CGG | CTG | GTG | GAG | AGC | CGG | GGG | CTG | ATG | AAG | GAG | CAG | 1372 |
| | Gly | Arg | Cys | Pro | Arg | Leu | Val | Glu | Ser | Arg | Gly | Leu | Met | Lys | Glu | Gln | |
| | | | | 395 | | | | | 400 | | | | | 405 | | | |
| 20 | TAC | GAG | GGC | AGG | CTG | GTG | CTG | CTC | ACC | GAG | CCG | GGC | AAC | GGC | ACC | TAC | 1420 |
| | Tyr | Glu | Gly | Arg | Leu | Val | Leu | Leu | Thr | Glu | Pro | Gly | Asn | Gly | Thr | Tyr | |
| | | | 410 | | | | | 415 | | | | | 420 | | | | |
| 25 | ACC | GTC | ATC | CTC | AAC | CAG | CTC | ACC | GAT | CAG | GAC | GCC | GGC | TTC | TAC | TGG | 1468 |
| | Thr | Val | Ile | Leu | Asn | Gln | Leu | Thr | Asp | Gln | Asp | Ala | Gly | Phe | Tyr | Trp | |
| | 425 | | | | | 430 | | | | | | 435 | | | | | |
| 30 | TGC | GTG | ACC | GAC | GGC | GAC | ACG | CGC | TGG | ATC | TCC | ACA | GTG | GAG | CTC | AAG | 1516 |
| | Cys | Val | Thr | Asp | Gly | Asp | Thr | Arg | Trp | Ile | Ser | Thr | Val | Glu | Leu | Lys | |
| | 440 | | | | | 445 | | | | | 450 | | | | | 455 | |
| 35 | GTT | GTC | CAA | GGA | GAA | CCA | AGC | CTC | AAG | GTA | CCC | AAG | AAC | GTC | ACG | GCT | 1564 |
| | Val | Val | Gln | Gly | Glu | Pro | Ser | Leu | Lys | Val | Pro | Lys | Asn | Val | Thr | Ala | |
| | | | | | 460 | | | | 465 | | | | | | 470 | | |
| 40 | TGG | CTG | GGA | GAG | CCC | TTA | AAG | CTC | TCC | TGC | CAC | TTC | CCC | TGC | AAA | TTC | 1612 |
| | Trp | Leu | Gly | Glu | Pro | Leu | Lys | Leu | Ser | Cys | His | Phe | Pro | Cys | Lys | Phe | |
| | | | | 475 | | | | 480 | | | | | | 485 | | | |
| 45 | TAC | TCC | TTT | GAG | AAG | TAC | TGG | TGT | AAG | TGG | AGC | AAC | AGA | GGC | TGC | AGC | 1660 |
| | Tyr | Ser | Phe | Glu | Lys | Tyr | Trp | Cys | Lys | Trp | Ser | Asn | Arg | Gly | Cys | Ser | |
| | | | 490 | | | | | 495 | | | | | 500 | | | | |
| 50 | GCC | CTG | CCC | ACC | CAG | AAC | GAC | GGC | CCC | AGC | CAG | GCC | TTT | GTG | AGC | TGC | 1708 |
| | Ala | Leu | Pro | Thr | Gln | Asn | Asp | Gly | Pro | Ser | Gln | Ala | Phe | Val | Ser | Cys | |
| | 505 | | | | | | 510 | | | | | 515 | | | | | |
| 55 | GAC | CAG | AAC | AGC | CAG | GTC | GTC | TCC | CTG | AAC | CTG | GAC | ACA | GTC | ACC | AAG | 1756 |
| | Asp | Gln | Asn | Ser | Gln | Val | Val | Ser | Leu | Asn | Leu | Asp | Thr | Val | Thr | Lys | |
| | 520 | | | | | 525 | | | | | 530 | | | | | 535 | |
| 60 | GAG | GAT | GAA | GGC | TGG | TAC | TGG | TGT | GGA | GTG | AAG | GAA | GGC | CCC | CGA | TAC | 1804 |
| | Glu | Asp | Glu | Gly | Trp | Tyr | Trp | Cys | Gly | Val | Lys | Glu | Gly | Pro | Arg | Tyr | |
| | | | | | 540 | | | | 545 | | | | | | 550 | | |
| 65 | GGG | GAG | ACG | GCG | GCT | GTC | TAC | GTG | GCA | GTG | GAG | AGC | AGG | GTG | AAG | GGG | 1852 |
| | Gly | Glu | Thr | Ala | Ala | Val | Tyr | Val | Ala | Val | Glu | Ser | Arg | Val | Lys | Gly | |
| | | | | 555 | | | | 560 | | | | | | 565 | | | |
| 70 | TCC | CAA | GGC | GCC | AAG | CAA | GTG | AAA | GCT | GCC | CCT | GCG | GGG | GCG | GCA | ATA | 1900 |
| | Ser | Gln | Gly | Ala | Lys | Gln | Val | Lys | Ala | Ala | Pro | Ala | Gly | Ala | Ala | Ile | |
| | | | | 570 | | | | 575 | | | | | | 580 | | | |
| 75 | CAG | TCG | AGG | GCC | GGG | GAG | ATC | CAG | AAC | AAA | GCC | CTT | CTG | GAC | CCC | AGC | 1948 |
| | Gln | Ser | Arg | Ala | Gly | Glu | Ile | Gln | Asn | Lys | Ala | Leu | Leu | Asp | Pro | Ser | |
| | 585 | | | | | | 590 | | | | | 595 | | | | | |

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|----|--|------|
| | TTT TTC GCA AAG GAA AGT GTG AAG GAC GCT GCT GGT GGA CCC GGA GCA | 1996 |
| | Phe Phe Ala Lys Glu Ser Val Lys Asp Ala Ala Gly Gly Pro Gly Ala | |
| | 600 605 610 615 | |
| 5 | CCT GCA GAT CCT GGC CGC CCT ACA GGA TAC AGC GGG AGC TCC AAA GCA | 2044 |
| | Pro Ala Asp Pro Gly Arg Pro Thr Gly Tyr Ser Gly Ser Ser Lys Ala | |
| | 620 625 630 | |
| 10 | CTG GTC TCC ACC CTG GTG CCC CTG GCC CTG GTC CTG GTC GCA GGG GTC | 2092 |
| | Leu Val Ser Thr Leu Val Pro Leu Ala Leu Val Leu Val Ala Gly Val | |
| | 635 640 645 | |
| 15 | GTG GCG ATC GGG GTG GTC CGA GCC CGG CAC AGG AAG AAC GTC GAC CGG | 2140 |
| | Val Ala Ile Gly Val Val Arg Ala Arg His Arg Lys Asn Val Asp Arg | |
| | 650 655 660 | |
| 20 | ATT TCA ATC AGG AGC TAC CGG ACA GAT ATC AGC ATG TCA GAC TTT GAG | 2188 |
| | Ile Ser Ile Arg Ser Tyr Arg Thr Asp Ile Ser Met Ser Asp Phe Glu | |
| | 665 670 675 | |
| 25 | AAC TCC AGG GAT TTT GAA GGA CGT GAC AAC ATG GGA GCC TCT CCA GAG | 2236 |
| | Asn Ser Arg Asp Phe Glu Gly Arg Asp Asn Met Gly Ala Ser Pro Glu | |
| | 680 685 690 695 | |
| 30 | GCC CAA GAG ACG TCT CTC GGA GGG AAG GAC GAG TTT GCC ACC ACT ACC | 2284 |
| | Ala Gln Glu Thr Ser Leu Gly Gly Lys Asp Glu Phe Ala Thr Thr Thr | |
| | 700 705 710 | |
| 35 | GAG GAC ACC GTG GAG AGC AAA GAA CCC AAG AAG GCA AAG AGG TCG TCC | 2332 |
| | Glu Asp Thr Val Glu Ser Lys Glu Pro Lys Lys Ala Lys Arg Ser Ser | |
| | 715 720 725 | |
| 40 | AAG GAG GAA GCC GAC GAG GCC TTC ACC ACC TTC CTC CTC CAG GCC AAA | 2380 |
| | Lys Glu Ala Asp Glu Ala Phe Thr Thr Phe Leu Leu Gln Ala Lys | |
| | 730 735 740 | |
| 45 | AAC CTG GCC TCC GCC GCA ACC CAG AAC GGC CCG ACA GAA GCC TAG ACGGAG | 2431 |
| | Asn Leu Ala Ser Ala Ala Thr Gln Asn Gly Pro Thr Glu Ala | |
| | 745 750 755 | |
| 50 | CCCTGGGCGC CCCTTCCCTC CGCACGTGGC AATCACGCTC CGAATCACGC TGATCCTCAG | 2491 |
| | GGCCCTCAGC TCGGGGGGCT CCACTGCCTG CACTCACACC CCGCCTAGGC TTCTCCTGTC | 2551 |
| 55 | TGTCCTCAGA GGGTGTGCTG GTTCCTTCTT GGTGGCATCC AAGCCTGGCT TACTTGTTC | 2611 |
| | TATTGGGGGT GAGGTGGTAC GAGGAGTTCC CACCTGCAGC TTATTGGAAC GAGAGAACTA | 2671 |
| 60 | AAGGTGTGGA GGAGAATTAA GATCGCAGAG GGGCCTCTCA GAAAGAAAAG GAGTGGGTGG | 2731 |
| | GGAGACAACC GCAGAAAGGG GGCCATTGAG CGCTTCCCTG TCCCCTTATT TGGGGATGTC | 2791 |
| | AGTGGAAATCC TCCCTTCCAC CCCATCTCTG CACCTCTCCA TCCCCACTCC ATTCCATCTT | 2851 |
| 65 | CTCTTCTTCT TTCCCTCATT AAAAATGTGC ATTTGGTTAC TACTAGATT CCAGGGACTC | 2911 |
| | TGCTAGACAC TGGGATAGGT AGGCCGCAAT CCCAGGCGGC AGCCTTCCGC AAACATCAAG | 2971 |
| | GAGCCCCTGG AGCCCACAGC ATCTCTTCAC GTGTACACTC ACTGACCTCT GCCTCTGCTG | 3031 |
| | GGAGAAATCA TAAAGGGTCT GCAGCCCTGA GGCCTTAGGG ATTATGTAAC ACAGGCATAC | 3091 |
| | ACACAAGGCA CCATCAACAC ATTCTTACCA TTTCACAGGT GAGAAAGCCG AGGTCTGAG | 3151 |
| | AGGTGGAGAG GTTTGCTCAG AGTCAGCAAG TGAGATGTAC GAGTCTCAAG CTAAAGATTT | 3211 |

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GACACCTGCT GTCCCTACAG GAGGGCCTCC TCTCTCCAGA TGAGACAGCA TTCCATAGGA 3271
 AGGAGAAGAA AAATGTAAAT AAGACTGGTC TTTCACAGGC CCCACATCAG GGAAGATACC 3331
 5 CCTTTCCTG TCTGTCACTC ACAGAGACCT AATAGGATAA GAGAATGGTC AACACTCAAA 3391
 CCCCCGAATG TGAAGAGTTC TAAGTGGAAA GGGAGGAAAA AGGGGGGATT TGATGGTGCC 3451
 AGGGAGGGGC TGATCTCCAA AGAACTAAGG TTTAAGTTT TTTGTTTTT TTTTCCTTC 3511
 10 TTCTAAGCTC TGCACTTCAA CTAGCATCTA TGAGCTGGCA CTTGCTAACA AATCAAAAAT 3571
 GTGAATTAAT TAATAATTAA AGACCATGAT TTCCTCCAAA AAAAAAAAAA AAAAAAAAAA 3630
 15

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 757 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear
 25 DESCRIPTION: Bovine Polyimmunoglobulin Receptor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

30 Met Ser Arg Leu Phe Leu Ala Cys Leu Leu Ala Ile Phe Pro Val Val
 1 5 10 15
 Ser Met Lys Ser Pro Ile Phe Gly Pro Glu Glu Val Ser Ser Val Glu
 20 25 30
 35 Gly Arg Ser Val Ser Ile Lys Cys Tyr Tyr Pro Pro Thr Ser Val Asn
 35 40 45
 Arg His Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Gln Gly Arg Cys
 50 55 60
 40 Thr Thr Leu Ile Ser Ser Glu Gly Tyr Val Ser Asp Asp Tyr Val Gly
 65 70 75 80
 Arg Ala Asn Leu Thr Asn Phe Pro Glu Ser Gly Thr Phe Val Val Asp
 45 85 90 95
 Ile Ser His Leu Thr His Lys Asp Ser Gly Arg Tyr Lys Cys Gly Leu
 100 105 110
 50 Gly Ile Ser Ser Arg Gly Leu Asn Phe Asp Val Ser Leu Glu Val Ser
 115 120 125
 Gln Asp Pro Ala Gln Ala Ser His Ala His Val Tyr Thr Ile Asp Leu
 130 135 140
 55 Gly Arg Thr Val Thr Ile Asn Cys Pro Phe Thr Arg Ala Asn Ser Glu
 145 150 155 160
 Lys Arg Lys Ser Leu Cys Lys Lys Thr Ile Gln Asp Cys Phe Gln Val
 60 165 170 175
 Val Asp Ser Thr Gly Tyr Val Ser Asn Ser Tyr Lys Asp Arg Ala His
 180 185 190
 65 Ile Ser Ile Leu Gly Thr Asn Thr Leu Val Phe Ser Val Val Ile Asn
 195 200 205

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Arg Val Lys Leu Ser Asp Ala Gly Met Tyr Val Cys Gln Ala Gly Asp
 210 215 220
 5 Asp Ala Lys Ala Asp Lys Ile Asn Ile Asp Leu Gln Val Leu Glu Pro
 225 230 235 240
 Glu Pro Glu Leu Val Tyr Gly Asp Leu Arg Ser Ser Val Thr Phe Asp
 245 250 255
 10 Cys Ser Leu Gly Pro Glu Val Ala Asn Val Pro Lys Phe Leu Cys Gln
 260 265 270
 Lys Lys Asn Gly Gly Ala Cys Asn Val Val Ile Asn Thr Leu Gly Lys
 275 280 285
 15 Lys Ala Gln Asp Phe Gln Gly Arg Ile Val Ser Val Pro Lys Asp Asn
 290 295 300
 20 Gly Val Phe Ser Val His Ile Thr Ser Leu Arg Lys Glu Asp Ala Gly
 305 310 315 320
 Arg Tyr Val Cys Gly Ala Gln Pro Glu Gly Glu Pro Gln Asp Gly Trp
 325 330 335
 25 Pro Val Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Thr Ala Ile Pro
 340 345 350
 Ala Ser Pro Ser Val Val Lys Gly Val Arg Gly Gly Ser Val Thr Val
 355 360 365
 30 Ser Cys Pro Tyr Asn Pro Lys Asp Ala Asn Ser Ala Lys Tyr Trp Cys
 370 375 380
 35 His Trp Glu Glu Ala Gln Asn Gly Arg Cys Pro Arg Leu Val Glu Ser
 385 390 395 400
 Arg Gly Leu Met Lys Glu Gln Tyr Glu Gly Arg Leu Val Leu Leu Thr
 405 410 415
 40 Glu Pro Gly Asn Gly Thr Tyr Thr Val Ile Leu Asn Gln Leu Thr Asp
 420 425 430
 Gln Asp Ala Gly Phe Tyr Trp Cys Val Thr Asp Gly Asp Thr Arg Trp
 435 440 445
 45 Ile Ser Thr Val Glu Leu Lys Val Val Gln Gly Glu Pro Ser Leu Lys
 450 455 460
 50 Val Pro Lys Asn Val Thr Ala Trp Leu Gly Glu Pro Leu Lys Leu Ser
 465 470 475 480
 Cys His Phe Pro Cys Lys Phe Tyr Ser Phe Glu Lys Tyr Trp Cys Lys
 485 490 495
 55 Trp Ser Asn Arg Gly Cys Ser Ala Leu Pro Thr Gln Asn Asp Gly Pro
 500 505 510
 Ser Gln Ala Phe Val Ser Cys Asp Gln Asn Ser Gln Val Val Ser Leu
 515 520 525
 60 Asn Leu Asp Thr Val Thr Lys Glu Asp Glu Gly Trp Tyr Trp Cys Gly
 530 535 540
 65 Val Lys Glu Gly Pro Arg Tyr Gly Glu Thr Ala Ala Val Tyr Val Ala
 545 550 555 560

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Val Glu Ser Arg Val Lys Gly Ser Gln Gly Ala Lys Gln Val Lys Ala
565 570 575

5 Ala Pro Ala Gly Ala Ala Ile Gln Ser Arg Ala Gly Glu Ile Gln Asn
580 585 590

Lys Ala Leu Leu Asp Pro Ser Phe Phe Ala Lys Glu Ser Val Lys Asp
595 600 605

10 Ala Ala Gly Gly Pro Gly Ala Pro Ala Asp Pro Gly Arg Pro Thr Gly
610 615 620

Tyr Ser Gly Ser Ser Lys Ala Leu Val Ser Thr Leu Val Pro Leu Ala
625 630 635 640

15 Leu Val Leu Val Ala Gly Val Val Ala Ile Gly Val Val Arg Ala Arg
645 650 655

20 His Arg Lys Asn Val Asp Arg Ile Ser Ile Arg Ser Tyr Arg Thr Asp
660 665 670

Ile Ser Met Ser Asp Phe Glu Asn Ser Arg Asp Phe Glu Gly Arg Asp
675 680 685

25 Asn Met Gly Ala Ser Pro Glu Ala Gln Glu Thr Ser Leu Gly Gly Lys
690 695 700

Asp Glu Phe Ala Thr Thr Thr Glu Asp Thr Val Glu Ser Lys Glu Pro
705 710 715 720

30 Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu Ala Asp Glu Ala Phe Thr
725 730 735

35 Thr Phe Leu Leu Gln Ala Lys Asn Leu Ala Ser Ala Ala Thr Gln Asn
740 745 750

Gly Pro Thr Glu Ala
755

40

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

45

(A) LENGTH: 3095 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50

DESCRIPTION: Mouse Polyimmunoglobulin Receptor

(ix) FEATURE:

55

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 85....2400

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

60 TCACCTGGAG AGAAGGAAGT AGCTAAAACA TTCTCATACA AGAAGCCAAC CTGAGCGGCA 60

CAGCCCCCCT GGAAGCCACA AGCA ATG AGG CTC TAC TTG TTC ACG CTC TTG 111

Met Arg Leu Tyr Leu Phe Thr Leu Leu

1 5

65 GTA ACT GTC TTT TCA GGG GTC TCC ACA AAA AGC CCC ATA TTT GGT CCC 159

Val Thr Val Phe Ser Gly Val Ser Thr Lys Ser Pro Ile Phe Gly Pro

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| | 10 | | 15 | | 20 | | 25 | |
|----|--|-----|-----|-----|-----|--|----|--|
| 5 | CAG GAG GTG AGT AGT ATA GAA GGC GAC TCT GTT TCC ATC ACG TGC TAC Gln Glu Val Ser Ser Ile Glu Gly Asp Ser Val Ser Ile Thr Cys Tyr | 30 | 35 | 40 | 207 | | | |
| 10 | TAC CCA GAC ACC TCT GTC AAC CGG CAC ACC CGG AAA TAC TGG TGC CGA Tyr Pro Asp Thr Ser Val Asn Arg His Thr Arg Lys Tyr Trp Cys Arg | 45 | 50 | 55 | 255 | | | |
| 15 | CAA GGA GCC AGC GGC ATG TGC ACA ACG CTC ATC TCT TCA AAT GGC TAC Gln Gly Ala Ser Gly Met Cys Thr Thr Leu Ile Ser Ser Asn Gly Tyr | 60 | 65 | 70 | 303 | | | |
| 20 | CTC TCC AAG GAG TAT TCA GGC AGA GCC AAC CTC ATC AAC TTC CCA GAG Leu Ser Lys Glu Tyr Ser Gly Arg Ala Asn Leu Ile Asn Phe Pro Glu | 75 | 80 | 85 | 351 | | | |
| 25 | AAC AAC ACA TTT GTG ATT AAC ATT GAG CAG CTC ACC CAG GAC GAC ACT Asn Asn Thr Phe Val Ile Asn Ile Glu Gln Leu Thr Gln Asp Asp Thr | 90 | 95 | 100 | 399 | | | |
| 30 | GGG AGC TAC AAG TGT GGC CTG GGT ACC AGT AAC CGA GGC CTG TCC TTC Gly Ser Tyr Lys Cys Gly Leu Gly Thr Ser Asn Arg Gly Leu Ser Phe | 110 | 115 | 120 | 447 | | | |
| 35 | GAT GTC AGC CTG GAG GTC AGC CAG GTT CCT GAG TTG CCG AGT GAC ACC Asp Val Ser Leu Glu Val Ser Gln Val Pro Glu Leu Pro Ser Asp Thr | 125 | 130 | 135 | 495 | | | |
| 40 | CAC GTC TAC ACA AAG GAC ATA GGC AGA AAT GTG ACC ATT GAA TGC CCT His Val Tyr Thr Lys Asp Ile Gly Arg Asn Val Thr Ile Glu Cys Pro | 140 | 145 | 150 | 543 | | | |
| 45 | TTC AAA AGG GAG AAT GTT CCC AGC AAG AAA TCC CTG TGT AAG AAG ACA Phe Lys Arg Glu Asn Val Pro Ser Lys Lys Ser Leu Cys Lys Lys Thr | 155 | 160 | 165 | 591 | | | |
| 50 | AAC CAG TCC TGC GAA CTT GTC ATT GAC TCT ACT GAG AAG GTG AAC CCC Asn Gln Ser Cys Glu Leu Val Ile Asp Ser Thr Glu Lys Val Asn Pro | 170 | 175 | 180 | 639 | | | |
| 55 | AGC TAT ATA GGC AGA GCA AAA CTT TTT ATG AAA GGG ACC GAC CTA ACT Ser Tyr Ile Gly Arg Ala Lys Leu Phe Met Lys Gly Thr Asp Leu Thr | 190 | 195 | 200 | 687 | | | |
| 60 | GTA TTC TAT GTC AAC ATT AGT CAC CTA ACG CAC AAT GAT GCT GGG CTG Val Phe Tyr Val Asn Ile Ser His Leu Thr His Asn Asp Ala Gly Leu | 205 | 210 | 215 | 735 | | | |
| 65 | TAC ATC TGC CAA GCT GGA GAA GGT CCT AGT GCT GAT AAG AAG AAT GTT Tyr Ile Cys Gln Ala Gly Glu Gly Pro Ser Ala Asp Lys Lys Asn Val | 220 | 225 | 230 | 783 | | | |
| 70 | GAC CTC CAG GTG CTA GCG CCT GAG CCA GAG CTG CTT TAT AAA GAC CTG Asp Leu Gln Val Leu Ala Pro Glu Pro Glu Leu Tyr Lys Asp Leu | 235 | 240 | 245 | 831 | | | |
| 75 | AGG TCC TCA GTG ACT TTT GAA TGT GAC CTG GGC CGT GAG GTG GCA AAC Arg Ser Ser Val Thr Phe Glu Cys Asp Leu Gly Arg Glu Val Ala Asn | 250 | 255 | 260 | 879 | | | |
| 80 | GAG GCC AAA TAT CTG TGC CGG ATG AAT AAG GAA ACC TGT GAT GTG ATC Glu Ala Lys Tyr Leu Cys Arg Met Asn Lys Glu Thr Cys Asp Val Ile | 270 | 275 | 280 | 927 | | | |

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| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | ATT | AAC | ACC | CTG | GGG | AAG | AGG | GAT | CCA | GAC | TTT | GAG | GGC | AGG | ATC | CTG | 975 |
| | Ile | Asn | Thr | Leu | Gly | Lys | Arg | Asp | Pro | Asp | Phe | Glu | Gly | Arg | Ile | Leu | |
| | | | | 285 | | | | | 290 | | | | | 295 | | | |
| 5 | ATA | ACC | CCC | AAG | GAT | GAC | AAT | GGC | CGC | TTC | AGT | GTG | TTG | ATC | ACA | GGC | 1023 |
| | Ile | Thr | Pro | Lys | Asp | Asp | Asn | Gly | Arg | Phe | Ser | Val | Leu | Ile | Thr | Gly | |
| | | | 300 | | | | | 305 | | | | | 310 | | | | |
| 10 | CTG | AGG | AAG | GAG | GAT | GCA | GGG | CAC | TAC | CAG | TGT | GGA | GCC | CAC | AGT | TCT | 1071 |
| | Leu | Arg | Lys | Glu | Asp | Ala | Gly | His | Tyr | Gln | Cys | Gly | Ala | His | Ser | Ser | |
| | | | 315 | | | | 320 | | | | | 325 | | | | | |
| 15 | GGT | TTG | CCT | CAA | GAA | GGC | TGG | CCC | ATC | CAG | ACT | TGG | CAA | CTC | TTT | GTC | 1119 |
| | Gly | Leu | Pro | Gln | Glu | Gly | Trp | Pro | Ile | Gln | Thr | Trp | Gln | Leu | Phe | Val | |
| | | | 330 | | | 335 | | | | | 340 | | | | | 345 | |
| 20 | AAT | GAA | GAG | TCT | ACC | ATT | CCC | AAT | CGT | CGC | TCT | GTT | GTG | AAG | GGA | GTC | 1167 |
| | Asn | Glu | Glu | Ser | Thr | Ile | Pro | Asn | Arg | Arg | Ser | Val | Val | Lys | Gly | Val | |
| | | | | | 350 | | | | 355 | | | | | | 360 | | |
| 25 | ACA | GGA | GGC | TCT | GTG | GCC | ATC | GCC | TGT | CCC | TAT | AAC | CCC | AAG | GAA | AGC | 1215 |
| | Thr | Gly | Gly | Ser | Val | Ala | Ile | Ala | Cys | Pro | Tyr | Asn | Pro | Lys | Glu | Ser | |
| | | | | 365 | | | | 370 | | | | | | 375 | | | |
| 30 | AGC | AGC | CTC | AAG | TAC | TGG | TGT | CGC | TGG | GAA | GGG | GAC | GGA | AAT | GGA | CAT | 1263 |
| | Ser | Ser | Leu | Lys | Tyr | Trp | Cys | Arg | Trp | Glu | Gly | Asp | Gly | Asn | Gly | His | |
| | | | 380 | | | | | 385 | | | | | 390 | | | | |
| 35 | TGC | CCC | GCG | CTT | GTG | GGG | ACC | CAG | GCC | CAG | GTG | CAA | GAA | GAG | TAT | GAA | 1311 |
| | Cys | Pro | Ala | Leu | Val | Gly | Thr | Gln | Ala | Gln | Val | Gln | Glu | Glu | Tyr | Glu | |
| | | 395 | | | | 400 | | | | | 405 | | | | | | |
| 40 | GGC | CGA | CTG | GCA | CTG | TTT | GAT | CAG | CCA | GGC | AAT | GGT | ACT | TAC | ACT | GTC | 1359 |
| | Gly | Arg | Leu | Ala | Leu | Phe | Asp | Gln | Pro | Gly | Asn | Gly | Thr | Tyr | Thr | Val | |
| | | 410 | | | | 415 | | | | | 420 | | | | | 425 | |
| 45 | ATC | CTC | AAC | CAG | CTC | ACC | ACC | GAG | GAT | GCT | GGC | TTC | TAT | TGG | TGT | CTT | 1407 |
| | Ile | Leu | Asn | Gln | Leu | Thr | Thr | Glu | Asp | Ala | Gly | Phe | Tyr | Trp | Cys | Leu | |
| | | | | 430 | | | | 435 | | | | | | | 440 | | |
| 50 | ACC | AAT | GGT | GAC | TCT | CGC | TGG | AGA | ACC | ACA | ATA | GAA | CTC | CAG | GTT | GCC | 1455 |
| | Thr | Asn | Gly | Asp | Ser | Arg | Trp | Arg | Thr | Thr | Ile | Glu | Leu | Gln | Val | Ala | |
| | | | | 445 | | | | 450 | | | | | | 455 | | | |
| 55 | GAA | GCT | ACA | AGG | GAG | CCA | AAC | CTT | GAG | GTG | ACG | CCA | CAG | AAC | GCA | ACA | 1503 |
| | Glu | Ala | Thr | Arg | Glu | Pro | Asn | Leu | Glu | Val | Thr | Pro | Gln | Asn | Ala | Thr | |
| | | | 460 | | | | 465 | | | | | | 470 | | | | |
| 60 | GCA | GTA | CTA | GGA | GAG | ACC | TTC | ACC | GTT | TCC | TGC | CAC | TAT | CCG | TGC | AAA | 1551 |
| | Ala | Val | Leu | Gly | Glu | Thr | Phe | Thr | Val | Ser | Cys | His | Tyr | Pro | Cys | Lys | |
| | | | 475 | | | | 480 | | | | | 485 | | | | | |
| 65 | TTC | TAC | TCC | CAG | GAG | AAA | TAC | TGG | TGC | AAG | TGG | AGC | AAC | AAG | GGT | TGC | 1599 |
| | Phe | Tyr | Ser | Gln | Glu | Lys | Tyr | Trp | Cys | Lys | Trp | Ser | Asn | Lys | Gly | Cys | |
| | | 490 | | | | 495 | | | | | 500 | | | | 505 | | |
| 70 | CAC | ATC | CTG | CCA | AGC | CAT | GAC | GAA | GGT | GCC | CGC | CAA | TCT | TCT | GTG | AGC | 1647 |
| | His | Ile | Leu | Pro | Ser | His | Asp | Glu | Gly | Ala | Arg | Gln | Ser | Ser | Val | Ser | |
| | | | | 510 | | | | 515 | | | | | | | 520 | | |
| 75 | TGC | GAC | CAG | AGC | AGC | CAG | CTG | GTC | TCC | ATG | ACC | CTG | AAC | CCG | GTC | AGT | 1695 |
| | Cys | Asp | Gln | Ser | Ser | Gln | Leu | Val | Ser | Met | Thr | Leu | Asn | Pro | Val | Ser | |
| | | | | 525 | | | | 530 | | | | | | 535 | | | |

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| | | |
|----|---|------|
| | AAG GAA GAT GAA GGC TGG TAC TGG TGT GGG GTA AAG CAA GGC CAG ACC | 1743 |
| | Lys Glu Asp Glu Gly Trp Tyr Trp Cys Gly Val Lys Gln Gly Gln Thr | |
| | 540 545 550 | |
| 5 | TAT GGA GAA ACT ACC GCC ATC TAT ATA GCA GTT GAA GAG AGG ACC AGA | 1791 |
| | Tyr Gly Glu Thr Thr Ala Ile Tyr Ile Ala Val Glu Glu Arg Thr Arg | |
| | 555 560 565 | |
| 10 | GGG TCA TCC CAT GTC AAC CCA ACA GAT GCA AAT GCA CGT GCC AAA GTC | 1839 |
| | Gly Ser Ser His Val Asn Pro Thr Asp Ala Asn Ala Arg Ala Lys Val | |
| | 570 575 580 585 | |
| 15 | GCT CTG GAA GAA GAG GTA GTG GAC TCC TCC ATC AGT GAA AAA GAG AAC | 1887 |
| | Ala Leu Glu Glu Val Val Asp Ser Ser Ile Ser Glu Lys Glu Asn | |
| | 590 595 600 | |
| | AAA GCC ATT CCA AAT CCC GGG CCT TTT GCC AAC GAA AGA GAG ATA CAG | 1935 |
| | Lys Ala Ile Pro Asn Pro Gly Pro Phe Ala Asn Glu Arg Glu Ile Gln | |
| | 605 610 615 | |
| 20 | AAT GTG AGA GAC CAA GCT CAG GAG AAC AGA GCA TCT GGG GAT GCT GGC | 1983 |
| | Asn Val Arg Asp Gln Ala Gln Glu Asn Arg Ala Ser Gly Asp Ala Gly | |
| | 620 625 630 | |
| 25 | AGT GCT GAT GGA CAA AGC AGG AGC TCC AGC TCC AAA GTG CTG TTC TCC | 2031 |
| | Ser Ala Asp Gly Gln Ser Arg Ser Ser Ser Ser Lys Val Leu Phe Ser | |
| | 635 640 645 | |
| 30 | ACC CTG GTG CCC CTG GGT CTG GTG CTG GCA GTG GGT GCT ATA GCT GTG | 2079 |
| | Thr Leu Val Pro Leu Gly Leu Val Leu Ala Val Gly Ala Ile Ala Val | |
| | 650 655 660 665 | |
| 35 | TGG GTG GCC AGA GTC CGA CAT CGG AAG AAT GTA GAC CGC ATG TCA ATC | 2127 |
| | Trp Val Ala Arg Val Arg His Arg Lys Asn Val Asp Arg Met Ser Ile | |
| | 670 675 680 | |
| | AGC AGC TAC AGG ACA GAC ATT AGC ATG GCA GAC TTC AAG AAC TCC AGA | 2175 |
| | Ser Ser Tyr Arg Thr Asp Ile Ser Met Ala Asp Phe Lys Asn Ser Arg | |
| | 685 690 695 | |
| 40 | GAT TTG GGA GGC AAT GAC AAC ATG GGG GCC TCT CCA GAC ACA CAG CAA | 2223 |
| | Asp Leu Gly Gly Asn Asp Asn Met Gly Ala Ser Pro Asp Thr Gln Gln | |
| | 700 705 710 | |
| 45 | ACA GTC ATC GAA GGA AAA GAT GAA ATC GTG ACT ACC ACG GAG TGC ACC | 2271 |
| | Thr Val Ile Glu Gly Lys Asp Glu Ile Val Thr Thr Thr Glu Cys Thr | |
| | 715 720 725 | |
| 50 | GCT GAG CCA GAA GAA TCC AAG AAA GCA AAA AGG TCA TCC AAG GAG GAA | 2319 |
| | Ala Glu Pro Glu Glu Ser Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu | |
| | 730 735 740 745 | |
| 55 | GCT GAC ATG GCC TAC TCG GCA TTC CTG CTT CAG TCC AGC ACC ATA GCT | 2367 |
| | Ala Asp Met Ala Tyr Ser Ala Phe Leu Leu Gln Ser Ser Thr Ile Ala | |
| | 750 755 760 | |
| | GCA CAG GTC CAC GAT GGT CCC CAG GAA GCC TAG GCAGTGCTGA CCACCCACCC | 2420 |
| | Ala Gln Val His Asp Gly Pro Gln Glu Ala | |
| | 765 770 | |
| 60 | TTGCCTGTGA CAATCAACTT GAGAATCACA CTGATCCGCT CGCAGCCCAC ACTCACCCAT | 2480 |
| | CACCTCCGCT CTTCCCTCCT GTCCTCAGAG GTGTGCTGGT TCCTTCTCTG GCCATGGAAG | 2540 |
| 65 | CCTGGCCTAG TTACGCCTGT TTAGGAGAGA GTGTGAGGCG TTCTTTTCTC TATGAAGAGA | 2600 |

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GTGAGGTGGA AATGAGGAGG AGGTGAACCT GAGAGACATC TCTGGAGGAA GAGGGTTGAG 2660
 AATAGGGGCT CGTTTCAGGA GAAAAGGCCA TTTGAATCTT CTTTATAACC ATATGATAGG 2720
 5 ATGTCAGCGT AACTCTTCTC TCCTCCATCT CTCCTTTCCT ATCCTCTTGA TTCAAACAAC 2780
 ACATCTGAGA ACTCACTAGG CTTCAAGTGC TACTAAATGC TGAGAGCCAG GCCACAATCT 2840
 10 TTCTATAAAT ATTACTGGAA GAGATGCCAT CTCCTCCCAG ATTCTGTCTT TTCATTAAGA 2900
 TAAGACATCA TTACCAGGCA TACCTCCTGC CTCTGTGCCT CATAGGCATA CACAAGCCAT 2960
 AAGGGCATCA TGATTTTCAG ATGAGAAGAG ATGTTTCTCA AGAGTGCCTA GTGAGATAGA 3020
 15 CTAGCGTCAA ACCAGATGTG GCAACTCCTG GCTCTTGGCC TACGATCTGT CTTCAAGAAA 3080
 AAAAAAAAAA AAAAA 3095

20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 771 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear

30

DESCRIPTION: Mouse Polyimmunoglobulin Receptor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Arg Leu Tyr Leu Phe Thr Leu Leu Val Thr Val Phe Ser Gly Val
 1 5 10 15
 35 Ser Thr Lys Ser Pro Ile Phe Gly Pro Gln Glu Val Ser Ser Ile Glu
 20 25 30
 40 Gly Asp Ser Val Ser Ile Thr Cys Tyr Tyr Pro Asp Thr Ser Val Asn
 35 40 45
 Arg His Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Ser Gly Met Cys
 50 55 60
 45 Thr Thr Leu Ile Ser Ser Asn Gly Tyr Leu Ser Lys Glu Tyr Ser Gly
 65 70 75 80
 Arg Ala Asn Leu Ile Asn Phe Pro Glu Asn Asn Thr Phe Val Ile Asn
 85 90 95
 50 Ile Glu Gln Leu Thr Gln Asp Asp Thr Gly Ser Tyr Lys Cys Gly Leu
 100 105 110
 55 Gly Thr Ser Asn Arg Gly Leu Ser Phe Asp Val Ser Leu Glu Val Ser
 115 120 125
 Gln Val Pro Glu Leu Pro Ser Asp Thr His Val Tyr Thr Lys Asp Ile
 130 135 140
 60 Gly Arg Asn Val Thr Ile Glu Cys Pro Phe Lys Arg Glu Asn Val Pro
 145 150 155 160
 Ser Lys Lys Ser Leu Cys Lys Lys Thr Asn Gln Ser Cys Glu Leu Val
 165 170 175

65

122

Ile Asp Ser Thr Glu Lys Val Asn Pro Ser Tyr Ile Gly Arg Ala Lys
 180 185 190
 5 Leu Phe Met Lys Gly Thr Asp Leu Thr Val Phe Tyr Val Asn Ile Ser
 195 200 205
 His Leu Thr His Asn Asp Ala Gly Leu Tyr Ile Cys Gln Ala Gly Glu
 210 215 220
 10 Gly Pro Ser Ala Asp Lys Lys Asn Val Asp Leu Gln Val Leu Ala Pro
 225 230 235 240
 Glu Pro Glu Leu Leu Tyr Lys Asp Leu Arg Ser Ser Val Thr Phe Glu
 245 250 255
 15 Cys Asp Leu Gly Arg Glu Val Ala Asn Glu Ala Lys Tyr Leu Cys Arg
 260 265 270
 20 Met Asn Lys Glu Thr Cys Asp Val Ile Ile Asn Thr Leu Gly Lys Arg
 275 280 285
 Asp Pro Asp Phe Glu Gly Arg Ile Leu Ile Thr Pro Lys Asp Asp Asn
 290 295 300
 25 Gly Arg Phe Ser Val Leu Ile Thr Gly Leu Arg Lys Glu Asp Ala Gly
 305 310 315 320
 His Tyr Gln Cys Gly Ala His Ser Ser Gly Leu Pro Gln Glu Gly Trp
 325 330 335
 30 Pro Ile Gln Thr Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile Pro
 340 345 350
 35 Asn Arg Arg Ser Val Val Lys Gly Val Thr Gly Gly Ser Val Ala Ile
 355 360 365
 Ala Cys Pro Tyr Asn Pro Lys Glu Ser Ser Ser Leu Lys Tyr Trp Cys
 370 375 380
 40 Arg Trp Glu Gly Asp Gly Asn Gly His Cys Pro Ala Leu Val Gly Thr
 385 390 395 400
 Gln Ala Gln Val Gln Glu Glu Tyr Glu Gly Arg Leu Ala Leu Phe Asp
 405 410 415
 45 Gln Pro Gly Asn Gly Thr Tyr Thr Val Ile Leu Asn Gln Leu Thr Thr
 420 425 430
 50 Glu Asp Ala Gly Phe Tyr Trp Cys Leu Thr Asn Gly Asp Ser Arg Trp
 435 440 445
 Arg Thr Thr Ile Glu Leu Gln Val Ala Glu Ala Thr Arg Glu Pro Asn
 450 455 460
 55 Leu Glu Val Thr Pro Gln Asn Ala Thr Ala Val Leu Gly Glu Thr Phe
 465 470 475 480
 Thr Val Ser Cys His Tyr Pro Cys Lys Phe Tyr Ser Gln Glu Lys Tyr
 485 490 495
 60 Trp Cys Lys Trp Ser Asn Lys Gly Cys His Ile Leu Pro Ser His Asp
 500 505 510
 65 Glu Gly Ala Arg Gln Ser Ser Val Ser Cys Asp Gln Ser Ser Gln Leu
 515 520 525

123

Val Ser Met Thr Leu Asn Pro Val Ser Lys Glu Asp Glu Gly Trp Tyr
530 535 540

5 Trp Cys Gly Val Lys Gln Gly Gln Thr Tyr Gly Glu Thr Thr Ala Ile
545 550 555 560

Tyr Ile Ala Val Glu Glu Arg Thr Arg Gly Ser Ser His Val Asn Pro
565 570 575

10 Thr Asp Ala Asn Ala Arg Ala Lys Val Ala Leu Glu Glu Glu Val Val
580 585 590

Asp Ser Ser Ile Ser Glu Lys Glu Asn Lys Ala Ile Pro Asn Pro Gly
595 600 605

15 Pro Phe Ala Asn Glu Arg Glu Ile Gln Asn Val Arg Asp Gln Ala Gln
610 615 620

20 Glu Asn Arg Ala Ser Gly Asp Ala Gly Ser Ala Asp Gly Gln Ser Arg
625 630 635 640

Ser Ser Ser Ser Lys Val Leu Phe Ser Thr Leu Val Pro Leu Gly Leu
645 650 655

25 Val Leu Ala Val Gly Ala Ile Ala Val Trp Val Ala Arg Val Arg His
660 665 670

Arg Lys Asn Val Asp Arg Met Ser Ile Ser Ser Tyr Arg Thr Asp Ile
675 680 685

30 Ser Met Ala Asp Phe Lys Asn Ser Arg Asp Leu Gly Gly Asn Asp Asn
690 695 700

Met Gly Ala Ser Pro Asp Thr Gln Gln Thr Val Ile Glu Gly Lys Asp
705 710 715 720

35 Glu Ile Val Thr Thr Thr Glu Cys Thr Ala Glu Pro Glu Glu Ser Lys
725 730 735

40 Lys Ala Lys Arg Ser Ser Lys Glu Glu Ala Asp Met Ala Tyr Ser Ala
740 745 750

Phe Leu Leu Gln Ser Ser Thr Ile Ala Ala Gln Val His Asp Gly Pro
755 760 765

45 Gln Glu Ala
770

(2) INFORMATION FOR SEQ ID NO: 9:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3269 base pairs
(B) TYPE: nucleic acid
55 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
DESCRIPTION: Rat Polyimmunoglobulin Receptor

(ix) FEATURE:

60

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 74....2383

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

65

GGCAACGAAG GTACCATGGA TCTTATACAA GAAGTGAACC AACATGCCGC AACCTCCTTG

60

124

| | | | | | | | | | | | | | | | | | |
|----|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | GAAGCCACAA | GCG | ATG | AGG | CTC | TCC | TTG | TTC | GCC | CTC | TTG | GTA | ACT | GTC | | 109 | |
| | | | Met | Arg | Leu | Ser | Leu | Phe | Ala | Leu | Leu | Val | Thr | Val | | | |
| | | | 1 | | | | 5 | | | | | 10 | | | | | |
| 5 | TTC | TCA | GGG | GTC | TCC | ACA | CAA | AGC | CCC | ATA | TTT | GGT | CCC | CAG | GAT | GTG | 157 |
| | Phe | Ser | Gly | Val | Ser | Thr | Gln | Ser | Pro | Ile | Phe | Gly | Pro | Gln | Asp | Val | |
| | | | 15 | | | | 20 | | | | | | 25 | | | | |
| 10 | AGT | AGT | ATT | GAA | GGT | AAC | TCG | GTC | TCC | ATC | ACG | TGC | TAC | TAC | CCA | GAC | 205 |
| | Ser | Ser | Ile | Glu | Gly | Asn | Ser | Val | Ser | Ile | Thr | Cys | Tyr | Tyr | Pro | Asp | |
| | | | 30 | | | | 35 | | | | | 40 | | | | | |
| 15 | ACC | TCT | GTC | AAC | CGG | CAC | ACC | CGG | AAA | TAC | TGG | TGC | CGA | CAA | GGA | GCC | 253 |
| | Thr | Ser | Val | Asn | Arg | His | Thr | Arg | Lys | Tyr | Trp | Cys | Arg | Gln | Gly | Ala | |
| | | | 45 | | | 50 | | | | | 55 | | | | | 60 | |
| 20 | AAC | GGC | TAC | TGC | GCA | ACC | CTC | ATC | TCT | TCA | AAT | GGC | TAC | CTC | TCG | AAG | 301 |
| | Asn | Gly | Tyr | Cys | Ala | Thr | Leu | Ile | Ser | Ser | Asn | Gly | Tyr | Leu | Ser | Lys | |
| | | | | | 65 | | | | | 70 | | | | | 75 | | |
| | GAG | TAT | TCA | GGC | AGA | GCC | AGC | CTC | ATC | AAC | TTC | CCA | GAG | AAT | AGC | ACA | 349 |
| | Glu | Tyr | Ser | Gly | Arg | Ala | Ser | Leu | Ile | Asn | Phe | Pro | Glu | Asn | Ser | Thr | |
| | | | | 80 | | | | 85 | | | | | | 90 | | | |
| 25 | TTT | GTG | ATT | AAC | ATT | GCA | CAT | CTC | ACC | CAG | GAG | GAC | ACT | GGG | AGC | TAC | 397 |
| | Phe | Val | Ile | Asn | Ile | Ala | His | Leu | Thr | Gln | Glu | Asp | Thr | Gly | Ser | Tyr | |
| | | | 95 | | | | | 100 | | | | | 105 | | | | |
| 30 | AAG | TGT | GGT | CTG | GGT | ACC | ACT | AAC | CGA | GGC | CTG | TTT | TTC | GAT | GTC | AGC | 445 |
| | Lys | Cys | Gly | Leu | Gly | Thr | Thr | Asn | Arg | Gly | Leu | Phe | Phe | Asp | Val | Ser | |
| | | | 110 | | | | 115 | | | | | 120 | | | | | |
| 35 | CTG | GAG | GTC | AGC | CAG | GTT | CCT | GAG | TTC | CCA | AAT | GAC | ACC | CAT | GTC | TAC | 493 |
| | Leu | Glu | Val | Ser | Gln | Val | Pro | Glu | Phe | Pro | Asn | Asp | Thr | His | Val | Tyr | |
| | | | 125 | | | 130 | | | | | 135 | | | | | 140 | |
| 40 | ACA | AAG | GAC | ATA | GGC | AGA | ACT | GTG | ACC | ATC | GAA | TGC | CGT | TTC | AAA | GAG | 541 |
| | Thr | Lys | Asp | Ile | Gly | Arg | Thr | Val | Thr | Ile | Glu | Cys | Arg | Phe | Lys | Glu | |
| | | | | | 145 | | | | | 150 | | | | | 155 | | |
| | GGG | AAT | GCT | CAT | AGC | AAG | AAA | TCC | CTG | TGT | AAG | AAG | AGA | GGA | GAG | GCC | 589 |
| | Gly | Asn | Ala | His | Ser | Lys | Lys | Ser | Leu | Cys | Lys | Lys | Arg | Gly | Glu | Ala | |
| | | | | 160 | | | | 165 | | | | | | 170 | | | |
| 45 | TGC | GAA | GTT | GTC | ATC | GAC | TCT | ACT | GAG | TAC | GTG | GAC | CCC | AGC | TAT | AAG | 637 |
| | Cys | Glu | Val | Val | Ile | Asp | Ser | Thr | Glu | Tyr | Val | Asp | Pro | Ser | Tyr | Lys | |
| | | | | 175 | | | | 180 | | | | | 185 | | | | |
| 50 | GAC | AGA | GCA | ATC | CTT | TTT | ATG | AAA | GGG | ACC | AGC | CGC | GAT | ATA | TTC | TAT | 685 |
| | Asp | Arg | Ala | Ile | Leu | Phe | Met | Lys | Gly | Thr | Ser | Arg | Asp | Ile | Phe | Tyr | |
| | | | 190 | | | | 195 | | | | | 200 | | | | | |
| 55 | GTC | AAC | ATT | AGC | CAC | CTA | ATA | CCC | AGT | GAT | GCT | GGA | CTG | TAT | GTT | TGC | 733 |
| | Val | Asn | Ile | Ser | His | Leu | Ile | Pro | Ser | Asp | Ala | Gly | Leu | Tyr | Val | Cys | |
| | | | 205 | | | 210 | | | | | 215 | | | | | 220 | |
| 60 | CAA | GCT | GGA | GAA | GGC | CCC | AGT | GCT | GAT | AAA | AAT | AAT | GCT | GAC | CTC | CAG | 781 |
| | Gln | Ala | Gly | Glu | Gly | Pro | Ser | Ala | Asp | Lys | Asn | Asn | Ala | Asp | Leu | Gln | |
| | | | | 225 | | | | | | 230 | | | | | 235 | | |
| | GTG | CTA | GAG | CCT | GAG | CCA | GAG | CTG | CTT | TAT | AAA | GAC | CTG | AGG | TCC | TCA | 829 |
| | Val | Leu | Glu | Pro | Glu | Pro | Glu | Leu | Leu | Tyr | Lys | Asp | Leu | Arg | Ser | Ser | |
| | | | | 240 | | | | 245 | | | | | | 250 | | | |

125

| | | |
|----|---|------|
| | GTG ACT TTT GAA TGT GAC CTG GGC CGT GAA GTG GCA AAT GAT GCC AAA | 877 |
| | Val Thr Phe Glu Cys Asp Leu Gly Arg Glu Val Ala Asn Asp Ala Lys | |
| | 255 260 265 | |
| 5 | TAT CTG TGT CGG AAG AAC AAG GAA ACC TGT GAT GTC ATC ATC AAC ACC | 925 |
| | Tyr Leu Cys Arg Lys Asn Lys Glu Thr Cys Asp Val Ile Ile Asn Thr | |
| | 270 275 280 | |
| 10 | CTG GGG AAG AGA GAT CCA GCC TTT GAA GGC AGG ATC CTG CTA ACC CCC | 973 |
| | Leu Gly Lys Arg Asp Pro Ala Phe Glu Gly Arg Ile Leu Leu Thr Pro | |
| | 285 290 295 300 | |
| 15 | AGG GAT GAC AAT GGC CGC TTC AGT GTG TTG ATC ACA GGC CTG AGG AAG | 1021 |
| | Arg Asp Asp Asn Gly Arg Phe Ser Val Leu Ile Thr Gly Leu Arg Lys | |
| | 305 310 315 | |
| 20 | GAG GAT GCA GGG CAC TAC CAG TGT GGA GCG CAC AGT TCT GGT TTG CCT | 1069 |
| | Glu Asp Ala Gly His Tyr Gln Cys Gly Ala His Ser Ser Gly Leu Pro | |
| | 320 325 330 | |
| 25 | CAA GAA GGC TGG CCC GTC CAG GCT TGG CAA CTC TTT GTC AAT GAA GAG | 1117 |
| | Gln Glu Gly Trp Pro Val Gln Ala Trp Gln Leu Phe Val Asn Glu Glu | |
| | 335 340 345 | |
| 30 | TCC ACG ATT CCC AAT AGT CGC TCT GTT GTG AAG GGT GTC ACA GGA GGC | 1165 |
| | Ser Thr Ile Pro Asn Ser Arg Ser Val Val Lys Gly Val Thr Gly Gly | |
| | 350 355 360 | |
| 35 | TCT GTG GCC ATC GTC TGT CCC TAT AAC CCC AAG GAA AGC AGC AGC CTC | 1213 |
| | Ser Val Ala Ile Val Cys Pro Tyr Asn Pro Lys Glu Ser Ser Ser Leu | |
| | 365 370 375 380 | |
| 40 | AAG TAC TGG TGT CAC TGG GAA GCC GAC GAG AAT GGA CGC TGC CCG GTG | 1261 |
| | Lys Tyr Trp Cys His Trp Glu Ala Asp Glu Asn Gly Arg Cys Pro Val | |
| | 385 390 395 | |
| 45 | CTC GTG GGG ACC CAG GCC CTG GTG CAA GAA GGA TAT GAA GGC CGA CTG | 1309 |
| | Leu Val Gly Thr Gln Ala Leu Val Gln Glu Gly Tyr Glu Gly Arg Leu | |
| | 400 405 410 | |
| 50 | GCA CTG TTC GAT CAG CCG GGC AGT GGC GCC TAC ACT GTC ATC CTC AAC | 1357 |
| | Ala Leu Phe Asp Gln Pro Gly Ser Gly Ala Tyr Thr Val Ile Leu Asn | |
| | 415 420 425 | |
| 55 | CAG CTC ACC ACC CAG GAT TCT GGC TTC TAC TGG TGT CTT ACC GAT GGT | 1405 |
| | Gln Leu Thr Thr Gln Asp Ser Gly Phe Tyr Trp Cys Leu Thr Asp Gly | |
| | 430 435 440 | |
| 60 | GAC TCT CGC TGG AGA ACC ACG ATA GAA CTG CAG GTT GCT GAA GCT ACA | 1453 |
| | Asp Ser Arg Trp Arg Thr Thr Ile Glu Leu Gln Val Ala Glu Ala Thr | |
| | 445 450 455 460 | |
| 65 | AAG AAG CCA GAC CTT GAG GTG ACA CCA CAG AAC GCG ACC GCG GTG ATA | 1501 |
| | Lys Lys Pro Asp Leu Glu Val Thr Pro Gln Asn Ala Thr Ala Val Ile | |
| | 465 470 475 | |
| 70 | GGA GAG ACC TTC ACA ATC TCC TGC CAC TAT CCG TGC AAA TTC TAC TCC | 1549 |
| | Gly Glu Thr Phe Thr Ile Ser Cys His Tyr Pro Cys Lys Phe Tyr Ser | |
| | 480 485 490 | |
| 75 | CAG GAG AAA TAC TGG TGC AAG TGG AGC AAC GAC GGC TGC CAC ATC CTG | 1597 |
| | Gln Glu Lys Tyr Trp Cys Lys Trp Ser Asn Asp Gly Cys His Ile Leu | |
| | 495 500 505 | |

126

| | | |
|----|---|------|
| | CCG AGC CAT GAT GAA GGT GCC CGC CAG TCC TCT GTG AGC TGT GAC CAG | 1645 |
| | Pro Ser His Asp Glu Gly Ala Arg Gln Ser Ser Val Ser Cys Asp Gln | |
| | 510 515 520 | |
| 5 | AGC AGC CAG ATC GTC TCC ATG ACC CTG AAC CCG GTC AAA AAG GAA GAT | 1693 |
| | Ser Ser Gln Ile Val Ser Met Thr Leu Asn Pro Val Lys Lys Glu Asp | |
| | 525 530 535 540 | |
| 10 | GAA GGC TGG TAC TGG TGT GGG GTA AAA GAA GGT CAG GTC TAT GGA GAA | 1741 |
| | Glu Gly Trp Tyr Trp Cys Gly Val Lys Glu Gly Gln Val Tyr Gly Glu | |
| | 545 550 555 | |
| 15 | ACT ACA GCC ATC TAT GTA GCA GTT GAA GAG AGG ACC AGA GGG TCA CCC | 1789 |
| | Thr Thr Ala Ile Tyr Val Ala Val Glu Arg Thr Arg Gly Ser Pro | |
| | 560 565 570 | |
| 20 | CAC ATC AAC CCG ACA GAT GCA AAC GCA CGT GCA AAA GAT GCT CCA GAG | 1837 |
| | His Ile Asn Pro Thr Asp Ala Asn Ala Arg Ala Lys Asp Ala Pro Glu | |
| | 575 580 585 | |
| 25 | GAA GAG GCA ATG GAA TCC TCT GTC AGG GAG GAT GAA AAC AAG GCC AAT | 1885 |
| | Glu Glu Ala Met Glu Ser Ser Val Arg Glu Asp Glu Asn Lys Ala Asn | |
| | 590 595 600 | |
| 30 | CTG GAC CCC AGG CTT TTT GCA GAC GAA AGA GAG ATA CAG AAT GCG GGA | 1933 |
| | Leu Asp Pro Arg Leu Phe Ala Asp Glu Arg Glu Ile Gln Asn Ala Gly | |
| | 605 610 615 620 | |
| 35 | GAC CAA GCT CAG GAG AAC AGA GCA TCT GGG AAT GCT GGC AGT GCT GGT | 1981 |
| | Asp Gln Ala Gln Glu Asn Arg Ala Ser Gly Asn Ala Gly Ser Ala Gly | |
| | 625 630 635 | |
| 40 | GGA CAA AGC GGG AGC TCC AAA GTC CTA TTC TCC ACC CTG GTG CCC CTG | 2029 |
| | Gly Gln Ser Gly Ser Ser Lys Val Leu Phe Ser Thr Leu Val Pro Leu | |
| | 640 645 650 | |
| 45 | GGT TTG GTG CTG GCA GTG GGT GCT GTG GCT GTG TGG GTG GCC AGA GTC | 2077 |
| | Gly Leu Val Leu Ala Val Gly Ala Val Ala Val Trp Val Ala Arg Val | |
| | 655 660 665 | |
| 50 | CGA CAT CGG AAG AAT GTA GAC CGC ATG TCA ATC AGC AGC TAC AGG ACA | 2125 |
| | Arg His Arg Lys Asn Val Asp Arg Met Ser Ile Ser Ser Tyr Arg Thr | |
| | 670 675 680 | |
| 55 | GAC ATT AGC ATG GGA GAC TTC AGG AAC TCC AGG GAT TTG GGA GGC AAT | 2173 |
| | Asp Ile Ser Met Gly Asp Phe Arg Asn Ser Arg Asp Leu Gly Gly Asn | |
| | 685 690 695 700 | |
| 60 | GAC AAC ATG GGC GCC ACT CCA GAC ACA CAA GAA ACA GTC CTC GAA GGA | 2221 |
| | Asp Asn Met Gly Ala Thr Pro Asp Thr Gln Glu Thr Val Leu Glu Gly | |
| | 705 710 715 | |
| 65 | AAA GAT GAA ATA GAG ACT ACC ACC GAG TGT ACC ACC GAG CCA GAG GAA | 2269 |
| | Lys Asp Glu Ile Glu Thr Thr Thr Glu Cys Thr Thr Glu Pro Glu Glu | |
| | 720 725 730 | |
| 70 | TCC AAG AAA GCA AAA AGG TCA TCC AAG GAG GAA GCT GAC ATG GCC TAC | 2317 |
| | Ser Lys Lys Ala Lys Arg Ser Ser Lys Glu Glu Ala Asp Met Ala Tyr | |
| | 735 740 745 | |
| 75 | TCA GCA TTC CTG TTT CAG TCC AGC ACA ATA GCT GCG CAG GTC CAT GAT | 2365 |
| | Ser Ala Phe L u Phe Gln Ser Ser Thr Ile Ala Ala Gln Val His Asp | |
| | 750 755 760 | |

127

GGT CCC CAG GAA GCC TAG GCAGTGCTGA CCACCTACCC CTGCCTGTGA CAATCAACT 2422
 Gly Pro Gln Glu Ala
 765

5 TGAGAATCAC ATTGATCCAC TCGCAGCCCA CCCTCGCCCA TCACCCAGGC TCTTCCCTCC 2482
 TGTTCTCAGA GGTGTGCTGG TTCCTCCCTC AGTCGTGGAA GCCTGGCCTA CTTATGCCTG 2542
 10 TTTAGGAGAG AGCGTGAGGA GTTCTTTTGT CTGTTAAAGA GTAAGGTGGA AATGAGTTGA 2602
 GCCCAAGAGG TGTCTCTGAG AGACGAGGGT TCAGAGCAGG GGCTCATTTC AGGAGGAAGA 2662
 GCCATTTGAA GCCTCTTTAT ACACATATGC TAGGATGTCA GGATAGCTCT TCTCCTCCAT 2722
 15 CTCTCCTTTC TTCTCTTCTT GATTTCAGACA ACAGATCCGA AAACCTACTA GGCTTCCGGT 2782
 GTCTACTAAA TGCTGAGAGT CAGGCCACAG CCTTCTATA AACATCACTG GAAGAGACAC 2842
 CACCTCGTCC CAGATTCTGT CTTTTCCCTA AGCTATCAAT CATTACCGGG GATTCCCTTT 2902
 20 GCCTCTGCAC CTCATAGGCA ACAAAGAAA CATAAGTCCT GCAGTCTAAG GCATACCCAA 2962
 GCCATAAGGG CACCACGAGA CTCAGATGAG AAGAGATTTT TCTCCAGAGT ACTCAGTGAG 3022
 25 ATAGACTAGT GTCAAGCCAG ATGGGGCAAC TCCTGGCTCT TGGCCTGGGA CTGTCTTCA 3082
 AGATCTCTGC TCTTATTAGA GAAAGAACTT TAGCATGAGG AAAAGTAAGA GAAAACAAGT 3142
 TACATGGGCA TGGTGGTGTG CTCCTGCAAT CCCAATATTA AGAGGTATAA AAATAGGACC 3202
 30 AGAAGTTTAA AGTAATCCTT GGCTACCTAG TGAGTGTAAG GCCAGCCTGG AATCAATAAG 3262
 AGTTGGT 3269

35

(2) INFORMATION FOR SEQ ID NO: 10:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 770 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear

45 DESCRIPTION: Rat Polyimmunoglobulin Receptor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Arg Leu Ser Leu Phe Ala Leu Leu Val Thr Val Phe Ser Gly Val
 50 1 5 10 15
 Ser Thr Gln Ser Pro Ile Phe Gly Pro Gln Asp Val Ser Ser Ile Glu
 20 25 30
 55 Gly Asn Ser Val Ser Ile Thr Cys Tyr Tyr Pro Asp Thr Ser Val Asn
 35 40 45
 Arg His Thr Arg Lys Tyr Trp Cys Arg Gln Gly Ala Asn Gly Tyr Cys
 50 55 60
 60 Ala Thr Leu Ile Ser Ser Asn Gly Tyr Leu Ser Lys Glu Tyr Ser Gly
 65 70 75 80
 Arg Ala Ser Leu Ile Asn Phe Pro Glu Asn Ser Thr Phe Val Ile Asn
 65 85 90 95

128

Ile Ala His Leu Thr Gln Glu Asp Thr Gly Ser Tyr Lys Cys Gly Leu
 100 105 110
 5 Gly Thr Thr Asn Arg Gly Leu Phe Phe Asp Val Ser Leu Glu Val Ser
 115 120 125
 Gln Val Pro Glu Phe Pro Asn Asp Thr His Val Tyr Thr Lys Asp Ile
 130 135 140
 10 Gly Arg Thr Val Thr Ile Glu Cys Arg Phe Lys Glu Gly Asn Ala His
 145 150 155 160
 Ser Lys Lys Ser Leu Cys Lys Lys Arg Gly Glu Ala Cys Glu Val Val
 165 170 175
 15 Ile Asp Ser Thr Glu Tyr Val Asp Pro Ser Tyr Lys Asp Arg Ala Ile
 180 185 190
 20 Leu Phe Met Lys Gly Thr Ser Arg Asp Ile Phe Tyr Val Asn Ile Ser
 195 200 205
 His Leu Ile Pro Ser Asp Ala Gly Leu Tyr Val Cys Gln Ala Gly Glu
 210 215 220
 25 Gly Pro Ser Ala Asp Lys Asn Asn Ala Asp Leu Gln Val Leu Glu Pro
 225 230 235 240
 Glu Pro Glu Leu Leu Tyr Lys Asp Leu Arg Ser Ser Val Thr Phe Glu
 245 250 255
 30 Cys Asp Leu Gly Arg Glu Val Ala Asn Asp Ala Lys Tyr Leu Cys Arg
 260 265 270
 35 Lys Asn Lys Glu Thr Cys Asp Val Ile Ile Asn Thr Leu Gly Lys Arg
 275 280 285
 Asp Pro Ala Phe Glu Gly Arg Ile Leu Leu Thr Pro Arg Asp Asp Asn
 290 295 300
 40 Gly Arg Phe Ser Val Leu Ile Thr Gly Leu Arg Lys Glu Asp Ala Gly
 305 310 315 320
 His Tyr Gln Cys Gly Ala His Ser Ser Gly Leu Pro Gln Glu Gly Trp
 325 330 335
 45 Pro Val Gln Ala Trp Gln Leu Phe Val Asn Glu Glu Ser Thr Ile Pro
 340 345 350
 50 Asn Ser Arg Ser Val Val Lys Gly Val Thr Gly Gly Ser Val Ala Ile
 355 360 365
 Val Cys Pro Tyr Asn Pro Lys Glu Ser Ser Ser Leu Lys Tyr Trp Cys
 370 375 380
 55 His Trp Glu Ala Asp Glu Asn Gly Arg Cys Pro Val Leu Val Gly Thr
 385 390 395 400
 60 Gln Ala Leu Val Gln Glu Gly Tyr Glu Gly Arg Leu Ala Leu Phe Asp
 405 410 415
 Gln Pro Gly Ser Gly Ala Tyr Thr Val Ile Leu Asn Gln Leu Thr Thr
 420 425 430
 65 Gln Asp Ser Gly Phe Tyr Trp Cys Leu Thr Asp Gly Asp Ser Arg Trp
 435 440 445

129

Arg Thr Thr Ile Glu Leu Gln Val Ala Glu Ala Thr Lys Lys Pro Asp
 450 455 460
 5 Leu Glu Val Thr Pro Gln Asn Ala Thr Ala Val Ile Gly Glu Thr Phe
 465 470 475 480
 Thr Ile Ser Cys His Tyr Pro Cys Lys Phe Tyr Ser Gln Glu Lys Tyr
 485 490 495
 10 Trp Cys Lys Trp Ser Asn Asp Gly Cys His Ile Leu Pro Ser His Asp
 500 505 510
 Glu Gly Ala Arg Gln Ser Ser Val Ser Cys Asp Gln Ser Ser Gln Ile
 515 520 525
 15 Val Ser Met Thr Leu Asn Pro Val Lys Lys Glu Asp Glu Gly Trp Tyr
 530 535 540
 20 Trp Cys Gly Val Lys Glu Gly Gln Val Tyr Gly Glu Thr Thr Ala Ile
 545 550 555 560
 Tyr Val Ala Val Glu Glu Arg Thr Arg Gly Ser Pro His Ile Asn Pro
 565 570 575
 25 Thr Asp Ala Asn Ala Arg Ala Lys Asp Ala Pro Glu Glu Glu Ala Met
 580 585 590
 Glu Ser Ser Val Arg Glu Asp Glu Asn Lys Ala Asn Leu Asp Pro Arg
 595 600 605
 30 Leu Phe Ala Asp Glu Arg Glu Ile Gln Asn Ala Gly Asp Gln Ala Gln
 610 615 620
 Glu Asn Arg Ala Ser Gly Asn Ala Gly Ser Ala Gly Gly Gln Ser Gly
 625 630 635 640
 35 Ser Ser Lys Val Leu Phe Ser Thr Leu Val Pro Leu Gly Leu Val Leu
 645 650 655
 40 Ala Val Gly Ala Val Ala Val Trp Val Ala Arg Val Arg His Arg Lys
 660 665 670
 Asn Val Asp Arg Met Ser Ile Ser Ser Tyr Arg Thr Asp Ile Ser Met
 675 680 685
 45 Gly Asp Phe Arg Asn Ser Arg Asp Leu Gly Gly Asn Asp Asn Met Gly
 690 695 700
 50 Ala Thr Pro Asp Thr Gln Glu Thr Val Leu Glu Gly Lys Asp Glu Ile
 705 710 715 720
 Glu Thr Thr Thr Glu Cys Thr Thr Glu Pro Glu Glu Ser Lys Lys Ala
 725 730 735
 55 Lys Arg Ser Ser Lys Glu Glu Ala Asp Met Ala Tyr Ser Ala Phe Leu
 740 745 750
 Phe Gln Ser Ser Thr Ile Ala Ala Gln Val His Asp Gly Pro Gln Glu
 755 760 765
 60 Ala
 65

130

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 322 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 10 DESCRIPTION: Guy's 13 Kappa

(ix) FEATURE:

15 (A) NAME/KEY: Coding Sequence
 (B) LOCATION: 8....320

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTCGAGC GAC ATT GTG ATG ACC CAG TCT CCA GCA ATC ATG TCT GCA TCT 49
 Asp Ile Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
 20 1 5 10

CCA GGG GAG AAG GTC ACC ATA ACC TGC AGT GCC AGC TCA AGT GTA AGT 97
 Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser
 15 20 25 30

TAC ATG CAC TGG TTC CAG CAG AAG CCA GGC ACT TCT CCC AAA CTC TGG 145
 Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp
 25 35 40 45

CTT TAT AGC ACA TCC AAC CTG GCT TCT GGA GTC CCT GCT CGC TTC AGT 193
 Leu Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
 30 50 55 60

GGC AGT GGA TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC CGA ATG GAG 241
 Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu
 35 65 70 75

GCT GAA GAT GCT GCC ACT TAT TAC TGC CAT CAA AGG ACT AGT TAC CCG 289
 Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Thr Ser Tyr Pro
 40 80 85 90

TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA A TA 322
 Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile
 45 95 100 105

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 105 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear
 55 DESCRIPTION: Guy's 13 Kappa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asp Ile Val Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
 60 1 5 10 15

Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30

His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Leu Tyr
 65 35 40 45

131

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
 5 65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Thr Ser Tyr Pro Tyr Thr
 85 90 95

10 Phe Gly Gly Gly Thr Lys Leu Glu Ile
 100 105

15 (2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 402 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 DESCRIPTION: Guy's 13 Gamma 1

25 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 7...402

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CTCGAG ATG GAA TGG ACC TGG GTT TTT CTC TTC CTC CTG TCA GGA ACT 48
 Met Glu Trp Thr Trp Val Phe Leu Phe Leu Leu Ser Gly Thr
 1 5 10

35 GCA GGC GTC CAC TCT GGG GTC CAG CTT CAG CAG TCA GGA CCT GAC CTG 96
 Ala Gly Val His Ser Gly Val Gln Leu Gln Gln Ser Gly Pro Asp Leu
 15 20 25 30

40 GTG AAA CCT GGG GCC TCA GTG AAG ATA TCC TGC AAG GCT TCT GGA TAC 144
 Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
 35 40 45

45 ACA TTC ACT GAC TAC AAC ATA CAC TGG GTG AAG CAG AGC CGT GGA AAG 192
 Thr Phe Thr Asp Tyr Asn Ile His Trp Val Lys Gln Ser Arg Gly Lys
 50 55 60

50 AGC CTT GAG TGG ATT GGA TAT ATT TAT CCT TAC AAT GGT AAT ACT TAC 240
 Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Asn Thr Tyr
 65 70 75

TAC AAC CAG AAG TTC AAG AAC AAG GCC ACA TTG ACT GTA GAC AAT TCC 288
 Tyr Asn Gln Lys Phe Lys Asn Lys Ala Thr Leu Thr Val Asp Asn Ser
 80 85 90

55 TCC ACC TCA GCC TAC ATG GAG CTC CGC AGC CTG ACA TCT GAG GAC TCT 336
 Ser Thr Ser Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser
 95 100 105 110

60 GCA GTC TAT TAC TGT GCA ACC TAC TTT GAC TAC TGG GGC CAA GGC ACC 384
 Ala Val Tyr Tyr Cys Ala Thr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
 115 120 125

65 ACT CTC ACA GTC TCC TCA 402
 Thr Leu Thr Val Ser Ser
 130

132

(2) INFORMATION FOR SEQ ID NO: 14:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 amino acids
 (B) TYPE: amino acid
 (C) STRANDNESS: single
 (D) TOPOLOGY: linear
 DESCRIPTION: Guy's 13 Gamma 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

15

Met Glu Trp Thr Trp Val Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15

Val His Ser Gly Val Gln Leu Gln Gln Ser Gly Pro Asp Leu Val Lys
 20 25 30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

Thr Asp Tyr Asn Ile His Trp Val Lys Gln Ser Arg Gly Lys Ser Leu
 50 55 60

Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Asn Thr Tyr Tyr Asn
 65 70 75 80

Gln Lys Phe Lys Asn Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Thr
 85 90 95

Ser Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
 100 105 110

Tyr Tyr Cys Ala Thr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 115 120 125

Thr Val Ser Ser
 130

45 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

55

ACCAGATCTA TGGATGGAC CTGGGTTTTT C

31

60 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

133

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

5 CCCAAGCTTG GTTTTGGAGA TGGTTTCTC 30

10 (2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

20 GATAAGCTTG GTCCTACTCC TCCTCCTCCT A 31

25 (2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

35 AATCTCGAGT CAGTAGCAGA TGCCATCTCC 30

40 (2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
45 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

50 GGAAAGCTTT GTACATATGC AAGGCTTACA 30

Claims

1. An immunoglobulin comprising a protection protein in association with an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain.
5
2. The immunoglobulin of claim 1 further comprising an immunoglobulin derived light chain having at least a portion of an antigen binding domain associated with said immunoglobulin derived heavy chain.
10
3. The immunoglobulin of claim 1 or 2 further comprising a second immunoglobulin derived heavy chain having at least a portion of an antigen binding domain associated with said protection protein.
15
4. The immunoglobulin of claim 3 further comprising a second immunoglobulin derived light chain having at least a portion of an antigen binding domain bound to said second immunoglobulin derived heavy chain.
20
5. The immunoglobulin of claims 1-4 further comprising immunoglobulin J chain bound to at least one of said immunoglobulin derived heavy chains.
25
6. The immunoglobulin of claims 1-5 that is a therapeutic immunoglobulin.
7. The immunoglobulin of claim 6 wherein said therapeutic immunoglobulin binds to mucosal pathogen antigens.
30
8. The immunoglobulin of claim 7 that is capable of preventing dental caries.
35
9. The immunoglobulin of claims 1-8 wherein said antigen binding domain is capable of binding an antigen

from S. mutans serotypes c, e and f or *S.sobrinus* serotypes d and g.

10. The immunoglobulin of claim 1 wherein said
5 protection protein has an amino acid sequence which substantially corresponds to at least a portion of the amino acid residues 1 to 627 of the rabbit polyimmunoglobulin receptor and does not have an amino acid residue sequence corresponding to amino acid residues 628-755 of
10 the rabbit polyimmunoglobulin receptor.

11. The immunoglobulin of claim 1 wherein said protection protein has an amino acid sequence which substantially corresponds to at least a portion of the
15 amino acid residues 1 to 606 of the rabbit polyimmunoglobulin receptor and does not have an amino acid sequence corresponding to amino acid residues 628-755 of the rabbit polyimmunoglobulin receptor.

20 12. The immunoglobulin of claim 10 or 11 wherein said protection protein has an amino acid sequence which does not contain amino acid residues corresponding to amino acid residues 628 to 775 of the rabbit polyimmunoglobulin receptor and which does contain amino
25 acid residues which correspond to one or more of the following amino acid segments:

- a) amino acids corresponding to amino acid residues 21-43 of the rabbit polyimmunoglobulin receptor;
- b) amino acids corresponding to amino acid residues
30 1 - 118 of the rabbit polyimmunoglobulin receptor;
- c) amino acids corresponding to amino acid residues 119 - 223 of the rabbit polyimmunoglobulin receptor;
- d) amino acids corresponding to amino acid residues 224 - 332 of the rabbit polyimmunoglobulin receptor;
- 35 e) amino acids corresponding to amino acid residues 333 - 441 of the rabbit polyimmunoglobulin receptor;

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f) amino acids corresponding to amino acid residues 442 - 552 of the rabbit polyimmunoglobulin receptor;

g) amino acids corresponding to amino acid residues 553 - 606 or 553 - 627 of the rabbit poly-
5 immunoglobulin receptor.

13. The immunoglobulin of claim 1 wherein said protection protein has an amino acid sequence which does not contain amino acid residues of a polyimmunoglobulin
10 receptor of a species which are analogous to amino acid residues 628 to 755 of the rabbit polyimmunoglobulin receptor and which does contain amino acid residues from a polyimmunoglobulin receptor of a species which are analogous to one or more of the following amino acid
15 segments:

a) amino acids corresponding to amino acid residues 21 - 43 of the rabbit polyimmunoglobulin receptor;

b) amino acids corresponding to amino acid residues 1 - 118 of the rabbit polyimmunoglobulin receptor;

20 c) amino acids corresponding to amino acid residues 119 - 223 of the rabbit polyimmunoglobulin receptor;

d) amino acids corresponding to amino acid residues 224 - 332 of the rabbit polyimmunoglobulin receptor;

e) amino acids corresponding to amino acid residues 333 - 441 of the rabbit polyimmunoglobulin receptor;

f) amino acids corresponding to amino acid residues 442 - 552 of the rabbit polyimmunoglobulin receptor;

g) amino acids corresponding to amino acid residues 553 - 606 or 553 - 627 of the rabbit polyimmunoglob-
30 ulin receptor.

14. The immunoglobulin of claim 13 wherein said species is human.

35 15. The immunoglobulin of claim 1 wherein said protection protein includes the amino acid sequence of at least one of the domains selected from the group consist-

ing of the following portions of the rabbit polyimmunoglobulin receptor: domain I, domain II, domain III, domain IV, domain V, and amino acid residues 553 to 627 of domain VI; and does not have an amino acid sequence corresponding
5 to amino acid residues 628-755 of the rabbit polyimmunoglobulin receptor.

16. The immunoglobulin of claim 1 wherein said protection protein does not have any amino acid sequence
10 which corresponds to or is analogous to amino acid residues 628-755 of the rabbit polyimmunoglobulin receptor and which does include:

a) at least one domain which is from the polyimmunoglobulin receptor of a first animal and which is
15 analogous to at least a portion of the following amino acid segments of the rabbit polyimmunoglobulin receptor: domain I, domain II, domain III, domain IV, domain V, and amino acid residues 553 to 627 of domain VI;

b) at least one domain which is from the polyimmunoglobulin receptor of a second animal and which corresponds to or is analogous to the following amino acid
20 residue segments of the rabbit polyimmunoglobulin receptor: domain I, domain II, domain III, domain IV, domain V, and amino acid residues 553 to 627 of domain VI.

25

17. The immunoglobulin of claim 1 wherein said protection protein does not have any amino acid sequence which corresponds to or is analogous to amino acid residues 628-755 of the rabbit polyimmunoglobulin receptor and
30 which does include:

a) at least one amino acid segment which is from the polyimmunoglobulin receptor of a first animal and which is analogous to at least a portion of the following amino acid residue segments of the rabbit
35 polyimmunoglobulin receptor: domain I, domain II, domain III, domain IV, domain V, and amino acid residues 553 to 627 of domain VI;

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b) at least one amino acid segment which is from the polyimmunoglobulin receptor of a second animal and which corresponds to or is analogous to the following amino acid residue segments of the rabbit polyimmunoglobulin receptor: domain I, domain II, domain III, domain IV,
5 domain V, and amino acid residues 553 to 627 of domain VI.

18. The immunoglobulin of claim 16 wherein said first animal is a mammal and said second animal is a
10 rabbit.

19. The immunoglobulin of claim 16 wherein said first animal is a human and said second animal is a rabbit.

15

20. The immunoglobulin of claim 1 wherein said immunoglobulin derived heavy chain contains at least a portion of an IgM or IgA heavy chain of any subtype.

21. The immunoglobulin of claim 1 wherein said
20 immunoglobulin derived heavy chain is comprised of immunoglobulin domains from two different isotopes of immunoglobulin.

22. The immunoglobulin of claim 21 wherein said
25 immunoglobulin domains are selected from the group consisting of:

a) the C_H1 of a mouse IgG1 and the C_H2 and C_H3 of mouse IgA; and

b) the C_H1 and C_H2 of a mouse IgG1 and the C_H2 and
30 C_H3 of mouse IgA;

23. The immunoglobulin of claim 1 wherein said antigen binding domain substantially corresponds to the Guy's 13 heavy chain variable region.

35

24. The immunoglobulin of claim 2 wherein said antigen binding domain substantially corresponds to the Guy's 13 light chain variable region.

5 25. The immunoglobulin of claim 1 wherein said protection protein has a first amino acid sequence which substantially corresponds to at least a portion of the amino acid residues 1 to 606 or 1 to 627 of the rabbit polyimmunoglobulin receptor and has a second amino acid
10 residue sequence contiguous with said first amino acid sequence, wherein said second amino acid residue sequence does not have an amino acid residue sequence corresponding to the functional transmembrane segment of the rabbit polyimmunoglobulin receptor.

15

26. The immunoglobulin of claim 25 wherein said second amino acid residue sequence has an amino acid sequence which corresponds to amino acid residues 655 to 755 of a polyimmunoglobulin receptor.

20

27. The immunoglobulin of claim 25 wherein said second amino acid residue sequence is a portion of one or more of the following: an intracellular domain of a polyimmunoglobulin molecule, a domain of a member of the
25 immunoglobulin gene superfamily, an enzyme, a toxin, or a linker.

28. A eukaryotic cell containing an immunoglobulin of claims 1-24.

30 29. The eukaryotic cell of claim 28 wherein said eukaryotic cell is a plant cell.

30. The plant cell of claim 29 wherein said plant cell is part of a plant.

35

31. A eukaryotic cell containing a nucleotide sequence encoding a protection protein.

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32. The eukaryotic cell of claim 31 which also contains a second nucleotide sequence encoding at least one of the molecules selected from the group consisting of: an immunoglobulin derived heavy chain having at least
5 a portion of an antigen binding domain, an immunoglobulin derived light chain having at least a portion of an antigen binding domain, or an immunoglobulin J chain.

33. The eukaryotic cell of claim 32 wherein said
10 second nucleotide sequence encodes an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain; and which also contains a third nucleotide sequence encoding an immunoglobulin derived light chain having at least a portion of an antigen
15 binding domain.

34. The eukaryotic cell of claim 33 which also contains a fourth nucleotide sequence encoding an immunoglobulin J chain.
20

35. The eukaryotic cell of claims 31-34 wherein said eukaryotic cell is a plant cell.

36. A plant cell containing a nucleotide sequence
25 encoding a protection protein and a nucleotide sequence encoding an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain.

37. A eukaryotic cell containing a protection
30 protein.

38. The eukaryotic cell of claim 37 which also contains at least one additional molecule selected from the group consisting of: an immunoglobulin derived heavy
35 chain having at least a portion of an antigen binding domain, an immunoglobulin derived light chain having at

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least a portion of an antigen binding domain, or an immunoglobulin J chain.

39. The eukaryotic cell of claim 38 wherein said
5 additional molecule is an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain; and which also contains an immunoglobulin derived light chain having at least a portion of an antigen binding domain.

10

40. The eukaryotic cell of claim 37 which also contains an immunoglobulin J chain.

41. The eukaryotic cell of claims 37-40 wherein
15 said eukaryotic cell is a plant cell.

42. The plant cell of claims 29, 35, 36 and 41 wherein said plant cell is derived from a dicotyledonous or monocotyledonous plant.

20

43. The plant cell of claims 29, 35, 36 and 41 wherein said plant cell is derived from a solanaceous plant.

25 44. The plant cell of claims 29, 35, 36 and 41 wherein said plant cell is alfalfa cell.

45. The plant cell of claims 29, 35, 36 and 41 wherein said plant cell is derived from a tobacco plant.

30

46. The plant cell of claims 29, 35, 36 and 41 wherein said plant cell is part of a plant.

47. A composition comprising an immunoglobulin of
35 claims 1-24 and plant macromolecules.

48. The composition of claim 47 wherein the plant molecules are derived from a dicotyledonous, monocotyledonous, solanaceous, alfalfa or tobacco plant.

5 49. The composition of claim 47 wherein said plant molecules are ribulose biphosphate carboxylase, light harvesting complex, pigments, secondary metabolites or chlorophyll.

10 50. The composition of claim 47 wherein said immunoglobulin is present in a concentration of between 0.001% and 99% mass excluding water.

15 51. The composition of claim 47 wherein said plant macromolecules are present in a concentration of between 1% and 99% mass excluding water.

52. A method of producing an immunoglobulin of claims 1-24 comprising the steps of:

20 (a) introducing into a plant cell an expression vector containing a nucleotide sequence encoding a protection protein operably linked to a transcriptional promoter; and

25 (b) introducing into said plant cell an expression vector containing a nucleotide sequence encoding an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain operably linked to a transcriptional promoter.

30 53. The method of claim 52 further comprising the step of:

(c) introducing into said plant cell an expression vector containing a nucleotide sequence encoding an immunoglobulin derived light chain having at least a
35 portion of an antigen binding domain operably linked to a transcriptional promoter.

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54. The method of claims 52 or 53 further comprising the step of introducing into said plant cell an expression vector containing a nucleotide sequence encoding an immunoglobulin J chain operably linked to a transcriptional promoter.

55. The method of claims 52-54 wherein said immunoglobulin derived heavy chain is immunoglobulin alpha chain and said immunoglobulin derived light chain is an immunoglobulin kappa or lambda chain.

56. The method of claims 52-54 wherein said immunoglobulin derived heavy chain is comprised of portions of immunoglobulin alpha chain and immunoglobulin gamma chain.

57. The method of claims 52-54 wherein said plant cells are part of a plant.

58. The method of claim 56 further comprising growing said plant.

59. The method of claims 56 or 57 wherein said plant is a dicotyledonous, monocotyledonous, solanaceous, leguminous, alfalfa or tobacco plant.

60. The methods of claims 52-59 wherein said immunoglobulin derived heavy chain is a chimeric immunoglobulin heavy chain.

61. A method of producing a therapeutic immunoglobulin composition containing plant macromolecules, said method comprising the step of shearing under pressure a portion of a plant of claims 30 or 46 to produce a pulp containing a therapeutic immunoglobulin and plant macromolecules in an liquid derived from the apoplast or symplast of said plant and solid plant derived material.

62. The method of claim 61 further comprising the step of separating said solid plant derived material from said liquid.

5 63. The method of claim 61 or 62 wherein said portion of said plant is a leaf, stem, root, tuber, fruit or entire plant.

64. The method of claim 61 wherein said shearing is
10 accomplished by a mechanical device which releases liquid from the apoplast or symplast of said plant.

65. The method of claim 62 wherein said separation
15 is by centrifugation, settling, flocculation or filtration.

66. A method for producing an assembled immunoglobulin molecule having heavy, light and J chains and a protection protein comprising the steps of:

- 20 a) introducing into a eukaryotic cell nucleotide sequences operably linked for expression encoding:
- i) an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain,
 - ii) an immunoglobulin derived light chain
25 having at least a portion of an antigen binding domain,
 - iii) an immunoglobulin J chain, and
 - iv) a protection protein; and
- b) maintaining said cell under conditions allowing production and assembly of said immunoglobulin derived
30 heavy and light chains, said immunoglobulin J chain and said protection protein into an immunoglobulin molecule.

67. A method for producing an assembled immunoglobulin molecule having heavy, light and J chains and a
35 protection protein by maintaining under conditions allowing protein production and immunoglobulin assembly, a

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eukaryotic cell containing nucleotide sequences operably linked for expression encoding:

- i) an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain,
- 5 ii) an immunoglobulin derived light chain having at least a portion of an antigen binding domain,
- iii) an immunoglobulin J chain, and
- iv) a protection protein.

10 68. The method of claims 66-67 wherein said eukaryotic cell is a plant cell.

69. A method of making an immunoglobulin resistant to environmental conditions comprising the steps of :

- 15 a) operably linking a nucleotide sequence encoding at least a portion of the antigen binding domain derived from an immunoglobulin heavy chain to a nucleotide sequence encoding at least one domain derived from an immunoglobulin alpha heavy chain to form a nucleotide
- 20 sequence encoding a chimeric immunoglobulin heavy chain;
- b) expressing said nucleotide sequence encoding said chimeric immunoglobulin heavy chain to produce said chimeric immunoglobulin heavy chain in a eukaryotic cell which also contains at least one other molecule selected
- 25 from the group consisting of: a protection protein, an immunoglobulin derived light chain having at least a portion of an antigen binding domain and an immunoglobulin J chain; and
- thereby allowing the chimeric immunoglobulin heavy chain
- 30 to assemble with said at least one other molecule to form said immunoglobulin resistant to said environmental conditions.

70. The method of claim 69 wherein said other
35 molecule is a protection protein and said eukaryotic cell also contains an immunoglobulin derived light chain having

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at least a portion of an antigen binding domain and an immunoglobulin J chain.

71. A process for producing an immunoglobulin
5 resistant to environmental conditions by maintaining under conditions allowing protein production and immunoglobulin assembly a cell containing:
- a) a nucleotide sequence encoding a chimeric immunoglobulin heavy chain in which a nucleotide sequence
10 encoding at least a portion of an antigen binding domain derived from heavy chain is operably linked to a nucleotide sequence encoding at least one domain derived from an immunoglobulin alpha heavy chain; and
 - b) at least one other molecule selected from the
15 group consisting of: a protection protein, an immunoglobulin derived light chain having at least a portion of an antigen binding domain and an immunoglobulin J chain;
- 20 thereby allowing the chimeric immunoglobulin heavy chain to assemble with said at least one other molecule to form said immunoglobulin resistant to said environmental conditions.

- 25 72. The immunoglobulin of claims 50-52 wherein said eukaryotic cell is a plant cell.

73. A tetratransgenic organism comprised of cells containing four different transgenes each encoding a
30 different polypeptide of a multi-peptide molecule wherein at least one of each of said different polypeptides is associated together in said multi-peptide molecule.

74. The transgenic organism of claim 73 wherein at
35 least one of said four transgenes is a transgene encoding a protection protein.

75. The transgenic organism of claim 73 wherein at least one of said four transgenes is a transgene encoding an immunoglobulin derived heavy chain having at least a portion of an antigen binding domain.

5

76. The transgenic organism of claim 73 wherein at least one of said four transgenes is a transgene encoding an immunoglobulin derived light chain having at least a portion of an antigen binding domain.

10

77. The transgenic organism of claim 73 wherein at least one of said four transgenes is a transgene encoding an immunoglobulin J chain.

15

78. The transgenic organism of claim 73 wherein at least one of said four transgenes is a transgene encoding a chimeric immunoglobulin heavy chain.

79. The transgenic organism of claim 73 wherein said transgenic organism is a plant.

80. The transgenic organism of claim 73 wherein said transgenic organism is a mammal.

81. The immunoglobulin of claim 1 wherein said chimeric immunoglobulin heavy chain contains an immunoglobulin domain from one of the following immunoglobulin heavy chains: IgG, IgA, IgM, IgE, IgD; and also contains a protection protein-binding domain from IgA or IgM.

30

82. The immunoglobulin of claim 81 wherein said immunoglobulin heavy chains are human, rodent, rabbit, bovine, ovine, caprine, fowl, canine, feline or primate immunoglobulin heavy chains.

35

83. The immunoglobulin of claim 81 wherein said protection protein-binding domain is from the IgA of a

human, rodent, rabbit, bovine, ovine, canine, feline or primate.

84. The immunoglobulin of claim 81 wherein said
5 chimeric immunoglobulin heavy chain is comprised of immunoglobulin chains of mouse IgG1 and said protection protein-binding domain is from mouse IgA or IgM.

85. The immunoglobulin of claim 81 wherein said
10 chimeric immunoglobulin heavy chain is comprised of immunoglobulin domains of a human IgG, IgM, IgD or IgE and said protection protein-binding domain is from a human IgA or IgM.

1/1

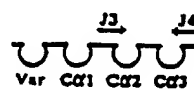
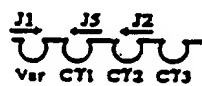
Synthetic oligonucleotide:

J1 ACCAGATCTTATGGAATGACCTGCGTTTTC
 J2 CCGAAGCTTGGTTTTCGAGATGTTTTC
 J3 GATAAGCTTGGTCTACTGCTGCTGCTA
 J4 AATCTCAATCAATGACAGATCCATCTCC
 J5 CCGAAGCTTGTACATATCGAAGCTTACA

Amplification by PCR:

Guy's 13

mopc 315.

Recombinant Heavy chains:

Plant G13



Plant G1/A



Plant G2/A



FIGURE 1

INTERNATIONAL SEARCH REPORT

Int: mal Application No
PCT/US 95/16889

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/13 C12N15/82 C07K16/00 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X Y | WO,A,91 16061 (HARVARD COLLEGE ;SUISSE RECH EXPERIMENT (CH)) 31 October 1991 --- -/-- | 1-15,20, 31,37 69-71 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *a* document member of the same patent family

Date of the actual completion of the international search

15 May 1996

Date of mailing of the international search report

04-06-96

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Maddox, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/16889

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | see page 43, line 14 - line 31 see page 45, line 26 - page 47, line 4 | 8,9, 21-24, 56,58, 69-71, 81-85 |
| Y | see page 52, line 8 - page 53, line 6 see page 55, line 3 - page 60, line 17 see page 67 - page 68 --- EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 24, 1994, pages 131-138, XP002003170 MA, J.K.-C., ET AL.: "Assembly of monoclonal antibodies with IgG1 and IgA heavy chain domains in transgenic tobacco plants" see page 137, left-hand column, paragraph 1 | 8,9, 21-24, 56,58, 69-71, 81-85 |
| X | --- FEBS LETTERS, vol. 307, no. 1, July 1992, page 71-75 XP002003171 HIATT, A., ET AL.: "Monoclonal antibody engineering in plants" see page 74 | 73 |
| P,X | --- SCIENCE, vol. 268, 5 May 1995, LANCASTER, PA US, pages 716-719, XP002003172 MA, J.K.-C., ET AL.: "Generation and assembly of secretory antibodies in plants" see the whole document | 1-85 |
| A | --- INFECTION AND IMMUNITY, vol. 62, no. 3, March 1994, pages 887-891, XP002003173 LEE, C.K., ET AL.: "Oral administration of polymeric immunoglobulin A prevents colonization with Vibrio cholerae in neonatal mice" see the whole document --- -/-- | 1-85 |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/16889

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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INTERNATIONAL SEARCH REPORT

Inter- national Application No
PCT/US 95/16889

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| | | CA-A- 2059289 | 17-10-91 |
| | | CN-A- 1057785 | 15-01-92 |
| | | EP-A- 0480014 | 15-04-92 |
| | | JP-T- 5501118 | 04-03-93 |
| | | OA-A- 9647 | 30-04-93 |
| ----- | | | |
| WO-A-9106320 | 16-05-91 | US-A- 5202422 | 13-04-93 |
| | | AU-B- 6753290 | 31-05-91 |
| | | CA-A- 2071502 | 28-04-91 |
| | | EP-A- 0497904 | 12-08-92 |
| | | JP-T- 5504333 | 08-07-93 |
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